

NOVEL COMPOUNDS

5 FIELD OF THE INVENTION

This invention relates to a novel group of 2,4,8-trisubstituted-8H-pyrido[2,3-d]pyrimidin-7-one compounds, processes for the preparation thereof, the use thereof in treating CSBP/p38 kinase mediated diseases and pharmaceutical compositions for use in such therapy.

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BACKGROUND OF THE INVENTION

Intracellular signal transduction is the means by which cells respond to extracellular stimuli. Regardless of the nature of the cell surface receptor (e. g. protein tyrosine kinase or seven-transmembrane G-protein coupled), protein kinases and phosphatases along with phospholipases are the essential machinery by which the signal is further transmitted within the cell [Marshall, J. C. Cell, **80**, 179-278 (1995)]. Protein kinases can be categorized into five classes with the two major classes being, tyrosine kinases and serine / threonine kinases depending upon whether the enzyme phosphorylates its substrate(s) on specific tyrosine(s) or serine / threonine(s) residues [Hunter, T., Methods in Enzymology (Protein Kinase Classification) p. 3, Hunter, T.; Sefton, B. M.; eds. vol. 200, Academic Press; San Diego, 1991].

For most biological responses, multiple intracellular kinases are involved and an individual kinase can be involved in more than one signaling event. These kinases are often cytosolic and can translocate to the nucleus or the ribosomes where they can affect transcriptional and translational events, respectively. The involvement of kinases in transcriptional control is presently much better understood than their effect on translation as illustrated by the studies on growth factor induced signal transduction involving MAP/ERK kinase [Marshall, C. J. Cell, **80**, 179 (1995); Herskowitz, I. Cell, **80**, 187 (1995); Hunter, T. Cell, **80**, 225 (1995); Seger, R., and Krebs, E. G. FASEB J., **7**, 726-735 (1995)].

While many signaling pathways are part of cell homeostasis, numerous cytokines (e.g., IL-1 and TNF) and certain other mediators of inflammation (e.g., COX-2, and iNOS) are produced only as a response to stress signals such as bacterial lipopolysaccharide (LPS). The first indications suggesting that the signal transduction pathway leading to LPS-induced cytokine biosynthesis involved protein kinases came from studies of Weinstein [Weinstein, *et al.*, J. Immunol. **151**, 3829(1993)] but the specific protein kinases involved were not identified. Working from a similar perspective, Han [Han, *et al.*, Science **265**, 808(1994)] identified murine p38 as a

kinase which is tyrosine phosphorylated in response to LPS. Definitive proof of the involvement of the p38 kinase in LPS-stimulated signal transduction pathway leading to the initiation of proinflammatory cytokine biosynthesis was provided by the independent discovery of p38 kinase by Lee [Lee; *et al.*, Nature, 372, 739(1994)] as the molecular target for a novel class of anti-inflammatory agents. The discovery of p38 (termed by Lee as CSBP 1 and 2) provided a mechanism of action of a class of anti-inflammatory compounds for which SK&F 86002 was the prototypic example. These compounds inhibited IL-1 and TNF synthesis in human monocytes at concentrations in the low uM range [Lee, *et al.*, Int. J. Immunopharmac. 10(7), 835(1988)] and exhibited activity in animal models which are refractory to cyclooxygenase inhibitors [Lee; *et al.*, Annals N. Y. Acad. Sci., 696, 149(1993)].

It is now firmly established that CSBP/p38 is a one of several kinases involved in a stress-response signal transduction pathway which is parallel to and largely independent of the analogous mitogen-activated protein kinase (MAP) kinase cascade. Stress signals, including LPS, pro-inflammatory cytokines, oxidants, UV light and osmotic stress, activate kinases upstream from CSBP/p38 which in turn phosphorylate CSBP/p38 at threonine 180 and tyrosine 182 resulting in CSBP/p38 activation. MAPKAP kinase-2 and MAPKAP kinase-3 have been identified as downstream substrates of CSBP/p38 which in turn phosphorylate heat shock protein Hsp 27 (Figure 1). Additional downstream substrates known to be phosphorylated by p38 include kinases (Mnk1/2, MSK1/2 and PRAK) and transcription factors (CHOP, MEF2, ATF2 and CREB). While many of the signaling pathways required for cytokine biosynthesis remain unknown it appears clear that many of the substrates for p38 listed above are involved. [Cohen, P. Trends Cell Biol., 353-361(1997) and Lee, J. C. *et al*, Pharmacol. Ther. vol. 82, nos. 2-3, pp. 389-397, 1999].

What is known, however, is that in addition to inhibiting IL-1 and TNF, CSBP/p38 kinase inhibitors (SK&F 86002 and SB 203580) also decrease the synthesis of a wide variety of pro-inflammatory proteins including, IL-6, IL-8, GM-CSF and COX-2. Inhibitors of CSBP/p38 kinase have also been shown to suppress the TNF-induced expression of VCAM-1 on endothelial cells, the TNF-induced phosphorylation and activation of cytosolic PLA2 and the IL-1-stimulated synthesis of collagenase and stromelysin. These and additional data demonstrate that CSBP/p38 is involved not only cytokine synthesis, but also in cytokine signaling [CSBP/P38 kinase reviewed in Cohen, P. Trends Cell Biol., 353-361(1997)].

Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) are biological substances produced by a variety of cells, such as monocytes or macrophages. IL-1 has been demonstrated to mediate a variety of biological activities thought to be important in immunoregulation and other physiological conditions such as

inflammation [See, e.g., Dinarello et al., Rev. Infect. Disease, 6, 51 (1984)]. The myriad of known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, neutrophil chemotaxis, induction of acute phase proteins and the suppression of plasma iron

5 levels.

There are many disease states in which excessive or unregulated IL-1 production is implicated in exacerbating and/or causing the disease. These include rheumatoid arthritis, osteoarthritis, endotoxemia and/or toxic shock syndrome, other acute or chronic inflammatory disease states such as the inflammatory reaction
10 induced by endotoxin or inflammatory bowel disease; tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's syndrome, rheumatoid arthritis, gout, traumatic arthritis, rubella arthritis, and acute synovitis. Evidence also links IL-1 activity to diabetes and pancreatic β cells [review of the biological activities which have been attributed to IL-1 Dinarello, J. Clinical Immunology, 5 (5), 287-297
15 (1985)].

Excessive or unregulated TNF production has been implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions; sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory
20 distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, such as influenza, cachexia secondary to infection or malignancy, cachexia, secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDS related complex),
25 keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyrexia.

Interleukin-8 (IL-8) is a chemotactic factor produced by several cell types including mononuclear cells, fibroblasts, endothelial cells, and keratinocytes. Its production from endothelial cells is induced by IL-1, TNF, or lipopolysaccharide (LPS). IL-8 stimulates a number of functions in vitro. It has been shown to have
30 chemoattractant properties for neutrophils, T-lymphocytes, and basophils. In addition it induces histamine release from basophils from both normal and atopic individuals as well as lysozymal enzyme release and respiratory burst from neutrophils. IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on neutrophils without de novo protein synthesis, this may contribute to increased
35 adhesion of the neutrophils to vascular endothelial cells. Many diseases are characterized by massive neutrophil infiltration. Conditions associated with an increased in IL-8 production (which is responsible for chemotaxis of neutrophil into

the inflammatory site) would benefit by compounds which are suppressive of IL-8 production.

IL-1 and TNF affect a wide variety of cells and tissues and these cytokines as well as other leukocyte derived cytokines are important and critical inflammatory mediators of a wide variety of disease states and conditions. The inhibition of these cytokines is of benefit in controlling, reducing and alleviating many of these disease states.

In addition to the involvement of CSBP/p38 signaling in the production of IL-1, TNF, IL-8, IL-6, GM-CSF, COX-2, collagenase and stromelysin, signal transduction via CSBP/p38 is required for the action of several of these same pro-inflammatory proteins plus many others (VEGF, PDGF, NGF) [Ono, K. and Han, J., Cellular Signalling, 12 1-13 (2000)]. The involvement of CSBP/p38 in multiple stress-induced signal transduction pathways provides additional rationale for the potential utility of CSBP/p38 in the treatment of diseases resulting from the excessive and destructive activation of the immune system. This expectation is supported by the potent and diverse activities described for CSBP/p38 kinase inhibitors [Badger, *et al.*, J. Pharm. Exp. Thera. 279 (3): 1453-1461.(1996); Griswold, *et al*, Pharmacol. Comm. 7, 323-229 (1996); Jackson, *et al.*, J. Pharmacol. Exp. Ther. 284, 687- 692 (1998); Underwood, *et al.*, J. Pharmacol. Exp. Ther. 293, 281- 288 (2000); Badger, *et al.*, Arthritis Rheum. 43, 175- 183 (2000)].

There remains a need for treatment, in this field, for compounds which are cytokine suppressive anti-inflammatory drugs, i.e. compounds which are capable of inhibiting the CSBP/p38/RK kinase.

Other pyrido[2,3-d]pyrimidine containing pharmacophores having varying pharmaceutical, insecticidal, and herbicidal activity may be found in the art, such as in WO 98/33798; WO 98/23613; WO 95/19774, now US Patent 6,265,410; WO 00/23444; WO 01/19828 (published after the filing date of this application); US 5,532,370; US 5,597,776; JP 2000 – 38350; WO 00/43374; WO 98/08846; and WO 01/55147 (also published after the filing date of this application).

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the p38 kinase cascade.

SUMMARY OF THE INVENTION

This invention relates to the novel compounds of Formula (I) and (Ia), and Formula (II) and (IIa), and pharmaceutical compositions comprising a compound of Formula (I) and (Ia), and Formula (II) and (IIa), and a pharmaceutically acceptable diluent or carrier.

This invention relates to a method of treating a CSBP/RK/p38 kinase mediated disease in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

This invention also relates to a method of inhibiting cytokines and the treatment of a cytokine mediated disease, in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

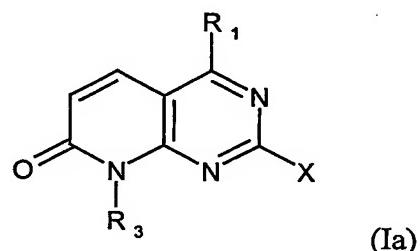
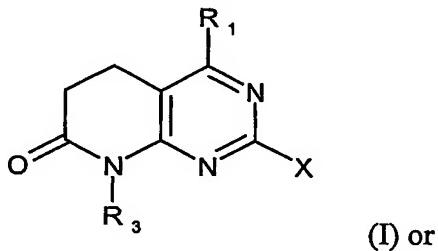
This invention more specifically relates to a method of inhibiting the production of IL-1 in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

This invention more specifically relates to a method of inhibiting the production of IL-6 in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

This invention more specifically relates to a method of inhibiting the production of IL-8 in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

This invention more specifically relates to a method of inhibiting the production of TNF in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) and (Ia), and Formula (II) and (IIa).

Accordingly, the present invention provides a compound of Formula (I) and (Ia):

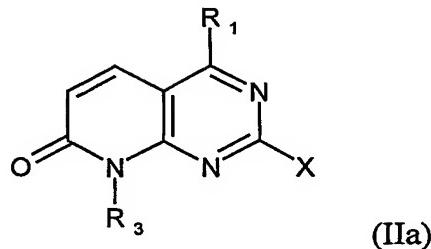
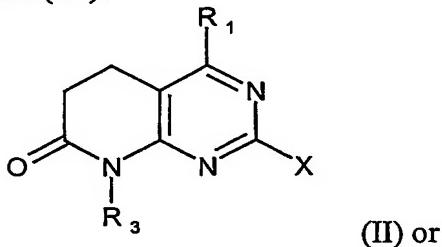


wherein

R₁ is an optionally substituted aryl or an optionally substituted heteroaryl ring;
R₂ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclC₁₋₁₀ alkyl moiety, which moieties are all optionally substituted, or R₂ is the moiety
5 X₁(CR₁₀R₂₀)_qC(A₁)(A₂)(A₃), or C(A₁)(A₂)(A₃);
A₁ is an optionally substituted C₁₋₁₀ alkyl;
A₂ is an optionally substituted C₁₋₁₀ alkyl;
A₃ is hydrogen or is an optionally substituted C₁₋₁₀ alkyl;
R₃ is an C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylC₁₋₄alkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclC₁₋₁₀ alkyl
10 moiety, which moieties are optionally substituted;
R₄ and R₁₄ are each independently selected from hydrogen, optionally substituted C₁₋₄ alkyl, optionally substituted C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylC₁₋₄alkyl, optionally substituted aryl, or optionally substituted aryl-C₁₋₄ alkyl, or R₄ and
15 R₁₄ together with the nitrogen which they are attached form an optionally substituted heterocyclic ring of 4 to 7 members, which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₉;
R₆ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocycl, heterocyclC₁₋₁₀alkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl or heteroarylC₁₋₁₀ alkyl, wherein each of these
20 moieties may be optionally substituted;
R₉ is hydrogen, C(Z)R₆ or optionally substituted C₁₋₁₀ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl;
R₁₀ and R₂₀ are independently selected from hydrogen or C₁₋₄alkyl;
X is R₂, OR₂, S(O)_mR₂, (CH₂)_nN(R₁₀)S(O)_mR₂, (CH₂)_nN(R₁₀)C(O)R₂,
25 (CH₂)_nNR₄R₁₄, or (CH₂)_nN(R₂)₂;
X₁ is N(R₁₀), O, S(O)_m, or CR₁₀R₂₀;
n is 0 or an integer having a value of 1 to 10;
m is 0 or an integer having a value of 1 or 2;
q is 0 or an integer having a value of 1 to 10;
30 Z is oxygen or sulfur;
or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

Another aspect of the present invention provides for the compound of Formula (II) and (IIa):



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wherein

R₁ is the moiety YRa;

R₂ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclylC₁₋₁₀ alkyl

10 moiety, which moieties are all optionally substituted, or R₂ is the moiety X₁(CR₁₀R₂₀)_q C(A₁)(A₂)(A₃), or C(A₁)(A₂)(A₃);

A₁ is an optionally substituted C₁₋₁₀ alkyl;

A₂ is an optionally substituted C₁₋₁₀ alkyl;

A₃ is hydrogen or is an optionally substituted C₁₋₁₀ alkyl;

15 R₃ is an C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylC₁₋₄alkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclylC₁₋₁₀ alkyl moiety, which moieties are optionally substituted;

R₄ and R₁₄ are each independently selected from hydrogen, optionally substituted C₁₋₄ alkyl, optionally substituted C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylC₁₋₄alkyl,

20 optionally substituted aryl, or optionally substituted aryl-C₁₋₄ alkyl, or R₄ and R₁₄ together with the nitrogen which they are attached form an optionally substituted heterocyclic ring of 4 to 7 members, which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₉;

R₆ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl, heterocyclyl C₁₋₁₀alkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl or heteroarylC₁₋₁₀ alkyl, wherein each of these moieties may be optionally substituted;

R₉ is hydrogen, C(Z)R₆ or optionally substituted C₁₋₁₀ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl;

R₁₀ and R₂₀ are independently selected from hydrogen or C₁₋₄alkyl;

30 Y is C(R_b)(R_d), C(O), N(R_d), N(R_d)C(R_c)(R_d), oxygen, OC(R_c)(R_d), S(O)m, or S(O)_mC(R_c)(R_d);

R_a is an aryl or heteroaryl ring, which ring is optionally substituted;

R_b is hydrogen, C₁₋₂ alkyl, NR_c, hydroxy, thio, C₁₋₂ alkoxy, S(O)_mC₁₋₂ alkyl;

- R_C is hydrogen or C₁₋₂ alkyl;
- R_d is hydrogen or C₁₋₂ alkyl;
- X is R₂, OR₂, S(O)_mR₂, (CH₂)_nN(R₁₀)S(O)_mR₂, (CH₂)_nN(R₁₀)C(O)R₂,
- (CH₂)_nNR₄R₁₄, or (CH₂)_nN(R₂)₂;
- 5 X₁ is N(R₁₀), O, S(O)_m, or CR₁₀R₂₀;
- n is 0 or an integer having a value of 1 to 10;
- m is 0 or an integer having a value of 1 or 2;
- q is 0 or an integer having a value of 1 to 10;
- Z is oxygen or sulfur;
- 10 or a pharmaceutically acceptable salt thereof.

The present invention is directed to novel compounds of Formula (I) and (Ia), and those of Formula (II) and (IIa), or a pharmaceutically acceptable salt thereof. As will be readily recognized, the difference between compounds of Formula (I) and (Ia) and that of Formula (II) and (IIa) lies in the unsaturation of the pyrido-7-one ring. The respective R₁, R₂, X and R₃ terms are the same for both groups within the 15 Formula itself, for instance I and Ia. For purposes herein, everything applicable to Formula (I) is also applicable to Formula (Ia) unless otherwise indicated, and everything applicable to Formula (II) is also applicable to Formula (IIa) unless otherwise indicated.

Suitably, for compounds of Formula (I), and (Ia), R₁ is an aryl, or heteroaryl ring, which ring is optionally substituted. The R₁ aryl or heteroaryl rings may be substituted one or more times, preferably 1 to 4 times, independently, by substituents selected from halogen, C₁₋₄ alkyl, halo-substituted-C₁₋₄ alkyl, cyano, nitro, (CR₁₀R₂₀)_vNR₄R₁₄, (CR₁₀R₂₀)_vC(Z)NR₄R₁₄, (CR₁₀R₂₀)_vC(Z)OR₈, (CR₁₀R₂₀)_vCOR_{a'}, (CR₁₀R₂₀)_vC(O)H, SR₅, S(O)R₅, S(O)₂R₅, (CR₁₀R₂₀)_vOR₈, ZC(Z)R₁₁, NR₁₀C(Z)R₁₁, or NR₁₀S(O)₂R₇.

Preferably, R₁ is an aryl moiety, more preferably a phenyl ring, optionally substituted one or more times by halogen, C₁₋₄ alkyl, or halo-substituted-C₁₋₄ alkyl. More preferably, the phenyl ring is substituted in the 2, 4, or 6-position, or di-substituted in the 2,4- position, such as 2-fluoro, 4-fluoro, 2,4-difluoro, or 2-methyl-4-fluoro; or tri-substituted in the 2,4,6-position such as 2,4,6-trifluoro. Another preferred embodiment is substitution of the phenyl ring in the 3-position, such as with a halogen derivative, producing a 3-position, 2,3-disubstitution, or a 3,4-disubstitution. Preferably, when R₁ is a heteroaryl moiety, the ring is not attached to the pharmacophore via one of the heteroatoms, such as nitrogen to form a charged ring. For instance, a pyridinyl ring would be attached through a carbon atom to yield a 2-, 3- or 4-pyridyl moiety, which is optionally substituted.

Suitably, v is 0 or an integer having a value of 1 or 2.

Suitably, Z is oxygen or sulfur.

Suitably, R_{a'} is C₁₋₄ alkyl, halo-substituted C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₃₋₇ cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylC₁₋₄ alkyl, heteroaryl, 5 heteroarylC₁₋₄ alkyl, heterocyclyl, heterocyclylC₁₋₄ alkyl, (CR₁₀R₂₀)_vOR₇, (CR₁₀R₂₀)_vS(O)_mR₇, (CR₁₀R₂₀)_vNR₁₀S(O)₂R₇, or (CR₁₀R₂₀)_vNR₄R₁₄; and wherein the aryl, arylalkyl, heteroaryl, heteroaryl alkyl may be optionally substituted.

Suitably, for compounds of Formula (II), and (IIa), R₁ is Y-R_a.

10 Suitably, Y is C(R_b)(R_d), C(O), N(R_d), N(R_d)C(R_c)(R_d), oxygen, OC(R_c)(R_d), S(O)_m, or S(O)_mC(R_c)(R_d).

Suitably, R_b is hydrogen, C₁₋₂ alkyl, NR_c, hydroxy, thio, C₁₋₂ alkoxy, S(O)_mC₁₋₂ alkyl.

Suitably, R_c is hydrogen or C₁₋₂ alkyl.

15 Suitably, R_d is hydrogen or C₁₋₂ alkyl.

Suitably, m is 0 or an integer having a value of 1 or 2.

Suitably R_a is an optionally substituted aryl ring or an optionally substituted heteroaryl ring. The optional substitutents for these rings are the same as for the Formula (I) and (Ia) R₁ aryl and heteroaryl rings as noted above.

20 As will be appreciated the difference between compounds of Formula (I) and (II) lies in the R₁ substitution. The remaining substituent groups are the same and for purposes herein applicable to all four formulas unless otherwise indicated.

Suitably, R₄ and R₁₄ are each independently selected from hydrogen, 25 optionally substituted C₁₋₄ alkyl, optionally substituted C₃₋₇cycloalkyl, optionally substituted C₃₋₇cycloalkylC₁₋₄ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl, or R₄ and R₁₄ together with the nitrogen to which they are attached may form an optionally substituted heterocyclic ring of 4 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₉.

30 The C₁₋₄ alkyl, C₃₋₇cycloalkyl, C₃₋₇cycloalkylC₁₋₄ alkyl, aryl and aryl-C₁₋₄ alkyl moieties may be optionally substituted, one or more times, preferably 1 to 4 times independently by halogen, such as fluorine, chlorine, bromine or iodine; hydroxy; hydroxy substituted C₁₋₁₀alkyl; C₁₋₁₀ alkoxy, such as methoxy or ethoxy; halosubstituted C₁₋₁₀ alkoxy; S(O)_m alkyl, such as methyl thio, methylsulfinyl or methyl sulfonyl; aldehydes (-C(O)), or a ketone, such as -C(O)R₆, such as C(O)C₁₋₁₀alkyl or C(O)aryl; amides, such as C(O)NR₄'R₁₄', or NR₄'C(O)C₁₋₁₀alkyl, or NR₄'C(O)aryl; NR₄'R₁₄', wherein R₄' and R₁₄' are each independently hydrogen or C₁₋₄ alkyl, or wherein the R₄'R₁₄' can cyclize together

- with the nitrogen to which they are attached to form a 5 to 7 membered ring which optionally contains an additional heteroatom selected from O/N/S; cyano, nitro, C₁₋₁₀ alkyl, C₃₋₇cycloalkyl, or C₃₋₇cycloalkyl C₁₋₁₀ alkyl group, such as methyl, ethyl, propyl, isopropyl, t-butyl, etc. or cyclopropyl methyl; halosubstituted C₁₋₁₀ alkyl, such as CF₂CF₂H, CH₂CF₃, or CF₃; an optionally substituted aryl, such as phenyl, or an optionally substituted arylalkyl, such as benzyl or phenethyl, wherein these aryl containing moieties may also be substituted one to two times by halogen; hydroxy; hydroxy substituted alkyl; C₁₋₁₀ alkoxy; S(O)malkyl; amino, mono & di-substituted C₁₋₄ alkyl amino, such as in the NR₄'R₁₄' group; C₁₋₄ alkyl, or CF₃.
- When R₄ and R₁₄ together with the nitrogen cyclize to form a ring, suitably, such rings include, but are not limited to pyrrolidine, piperidine, piperazine, morpholine, and thiomorpholine (including oxidizing the sulfur). The ring may be optional substituted, one or more times, preferably 1 to 4 times, independently by halogen, such as fluorine, chlorine, bromine or iodine; hydroxy; hydroxy substituted C₁₋₁₀alkyl; C₁₋₁₀ alkoxy, such as methoxy or ethoxy; halosubstituted C₁₋₁₀ alkoxy; S(O)m alkyl, such as methyl thio, methylsulfinyl or methyl sulfonyl; a ketone on the cyclized ring (-C(O)), or a ketone or aldehyde off the ring (-C(O)R₆), such as C(O)C₁₋₁₀ alkyl or C(O) aryl; NR₄'R₁₄', wherein R₄' and R₁₄' are each independently hydrogen or C₁₋₄ alkyl; C₁₋₁₀ alkyl, C₃₋₇cycloalkyl, or C₃₋₇cycloalkyl C₁₋₁₀ alkyl group, such as methyl, ethyl, propyl, isopropyl, t-butyl, etc. or cyclopropyl methyl; halosubstituted C₁₋₁₀ alkyl, such CF₂CF₂H, CH₂CF₃, or CF₃; an optionally substituted aryl, such as phenyl, or an optionally substituted arylalkyl, such as benzyl or phenethyl, wherein these aryl containing moieties may also be substituted one to two times by halogen; hydroxy; hydroxy substituted alkyl; C₁₋₁₀ alkoxy; S(O)malkyl; amino, mono & di-substituted C₁₋₄ alkyl amino, such as in the NR₄'R₁₄' group; C₁₋₄ alkyl, or CF₃.
- Suitably, R₅ is hydrogen, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl or NR₄R₁₄, excluding the moieties SR₅ being SNR₄R₁₄, S(O)₂R₅ being SO₂H and S(O)R₅ being SOH.
- Suitably, R₆ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl, heterocyclyl C₁₋₁₀alkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl or heteroarylC₁₋₁₀ alkyl, wherein these moieties may be optionally substituted.
- Suitably, R₇ is C₁₋₆alkyl, aryl, arylC₁₋₆alkyl, heterocyclic, heterocyclylC₁₋₆ alkyl, heteroaryl, or heteroarylC₁₋₆alkyl; and wherein each of these moieties may be optionally substituted.
- Suitably, R₈ is hydrogen, C₁₋₄ alkyl, halo-substituted C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₃₋₇ cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylC₁₋₄ alkyl, heteroaryl, heteroarylC₁₋₄ alkyl, heterocyclyl, heterocyclylC₁₋₄ alkyl,

(CR₁₀R₂₀)_tOR₇, (CR₁₀R₂₀)_tS(O)_mR₇, (CR₁₀R₂₀)_tNR₁₀S(O)₂R₇, or (CR₁₀R₂₀)_tNR₄R₁₄; and wherein the cycloalkyl, cycloalkenyl, aryl, arylalkyl, heteroaryl, heteroaryl alkyl, heterocyclic and heterocyclic alkyl moieties may be optionally substituted.

- 5 Suitably, t is an integer having a value of 1 to 3.
- Suitably, R₉ is hydrogen, C(Z)R₆, optionally substituted C₁₋₁₀ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl.
- Suitably, R₁₀ and R₂₀ are independently selected from hydrogen or a C₁₋₄ alkyl.
- 10 Suitably, R₁₁ is C₁₋₄ alkyl, halo-substituted C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₃₋₇ cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylC₁₋₄ alkyl, heteroaryl, heteroarylC₁₋₄ alkyl, heterocycl₁, heterocycl₁C₁₋₄ alkyl, (CR₁₀R₂₀)_tOR₇, (CR₁₀R₂₀)_tS(O)_mR₇, (CR₁₀R₂₀)_tNR₁₀S(O)₂R₇, or (CR₁₀R₂₀)_vNR₄R₁₄; and wherein the aryl, arylalkyl, heteroaryl, heteroaryl alkyl, heterocycl₁, and heterocycl₁alkyl moieties may be optionally substituted.
- 15 Suitably m is 0 or an integer having a value of 1 or 2.
- Suitably, R₃ is an optionally substituted C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylC₁₋₁₀alkyl, aryl, arylC₁₋₁₀ alkyl, heteroarylC₁₋₁₀alkyl, or heterocycl₁C₁₋₁₀ alkyl moiety, which moieties are optionally substituted one or more times,
- 20 preferably 1 to 4 times, independently by C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀alkynyl, C₃₋₇cycloalkyl, C₃₋₇cycloalkylC₁₋₁₀ alkyl, C₅₋₇cycloalkenyl, C₅₋₇cycloalkenylC₁₋₁₀ alkyl, halogen, cyano, nitro, (CR₁₀R₂₀)_nOR₆, (CR₁₀R₂₀)_nSH, (CR₁₀R₂₀)_nS(O)_mR₇, (CR₁₀R₂₀)_nNR₁₀S(O)₂R₇, (CR₁₀R₂₀)_nNR₄R₁₄, (CR₁₀R₂₀)_nCN,
- 25 (CR₁₀R₂₀)_nS(O)₂NR₄R₁₄, (CR₁₀R₂₀)_nC(Z)R₆, (CR₁₀R₂₀)_nOC(Z)R₆, (CR₁₀R₂₀)_nC(Z)OR₆, (CR₁₀R₂₀)_nC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z)R₆, (CR₁₀R₂₀)_nNR₁₀C(=NR₁₀)NR₄R₁₄, (CR₁₀R₂₀)_nOC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z)NR₄R₁₄, or (CR₁₀R₂₀)_nNR₁₀C(Z)OR₇.
- 30 Preferably the optional substituents are independently selected from halogen, alkyl, hydroxy, alkoxy, cyano, nitro, amino, or halosubstituted alkyl. More preferably, halogen, or alkyl.
- Preferably, R₃ is an optionally substituted C₁₋₁₀ alkyl, C₃₋₇cycloalkyl, C₃₋₇cycloalkylalkyl, or aryl. More preferably, R₃ is an optionally substituted C₁₋₁₀ alkyl, or aryl.
- 35 Preferably, when R₃ is an aryl moiety, it is a phenyl ring, optionally substituted one or more times by halogen, C₁₋₄ alkyl, or halo-substituted-C₁₋₄ alkyl. More preferably, the phenyl ring is substituted in the 2, 4, or 6-position, or di-substituted in

the 2,4- position, such as 2-fluoro, 4-fluoro, 2,4-difluoro, or 2-methyl-4-fluoro; or tri-substituted in the 2,4,6-position, such as 2,4,6-trifluoro.

Suitably, n is 0, or an integer having a value of 1 to 10.

Suitably, X is R₂, OR₂, S(O)_mR₂, (CH₂)_nN(R₁₀)S(O)_mR₂,

- 5 (CH₂)_nN(R₁₀)C(O)R₂, (CH₂)_nNR₄R₁₄, or (CH₂)_nN(R₂)₂. Preferably X is R₂, OR₂, (CH₂)_nNR₄R₁₄, or (CH₂)_nN(R₂)₂. Preferably, when X is R₂, then R₂ is the moiety X₁(CR₁₀R₂₀)_qC(A₁)(A₂)(A₃), or C(A₁)(A₂)(A₃).

Suitably, R₂ is independently selected from hydrogen, optionally substituted C₁₋₁₀ alkyl, optionally substituted C₃₋₇ cycloalkyl, optionally substituted C₃₋₇cycloalkylalkyl, optionally substituted aryl, optionally substituted arylC₁₋₁₀alkyl, optionally substituted heteroaryl, optionally substituted heteroarylC₁₋₁₀ alkyl, optionally substituted heterocyclic, optionally substituted heterocyclicC₁₋₁₀alkyl moiety, or R₂ is the moiety X₁(CR₁₀R₂₀)_qC(A₁)(A₂)(A₃), or C(A₁)(A₂)(A₃).

- The R₂ moieties, excluding hydrogen, may be optionally substituted one or more times, preferably 1 to 4 times, independently by C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇ cycloalkyl, C₃₋₇cycloalkylC₁₋₁₀alkyl, C₅₋₇cycloalkenyl, C₅₋₇ cycloalkenyl C₁₋₁₀ alkyl, halogen, -C(O), cyano, nitro, (CR₁₀R₂₀)_nOR₆, (CR₁₀R₂₀)_nSH, (CR₁₀R₂₀)_nS(O)_mR₇, (CR₁₀R₂₀)_nNR₁₀S(O)₂R₇, (CR₁₀R₂₀)_nNR₄R₁₄, (CR₁₀R₂₀)_nCN, (CR₁₀R₂₀)_nS(O)₂NR₄R₁₄, (CR₁₀R₂₀)_nC(Z)R₆, (CR₁₀R₂₀)_nOC(Z)R₆, (CR₁₀R₂₀)_nC(Z)OR₆, (CR₁₀R₂₀)_nC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z)R₆, (CR₁₀R₂₀)_nNR₁₀C(=NR₁₀)NR₄R₁₄, (CR₁₀R₂₀)_nC(=NOR₆)NR₄R₁₄, (CR₁₀R₂₀)_nOC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z)NR₄R₁₄, or (CR₁₀R₂₀)_nNR₁₀C(Z)OR₇.
- 25 Suitably X₁ is N(R₁₀), O, S(O)_m, or CR₁₀R₂₀. More preferably, X₁ is N(R₁₀), or O.

Suitably, q is 0 or an integer having a value of 1 to 10.

Suitably, A₁ is an optionally substituted C₁₋₁₀ alkyl.

Suitably, A₂ is an optionally substituted C₁₋₁₀ alkyl.

- 30 Suitably, A₃ is hydrogen or is an optionally substituted C₁₋₁₀ alkyl.
The A₁, A₂, and A₃ C₁₋₁₀ alkyl moieties may optionally substituted one or more times, independently, preferably from 1 to 4 times, with halogen, such as chlorine, fluorine, bromine, or iodine; halo-substituted C₁₋₁₀alkyl, such as CF₃, or CHF₂CF₃; C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇ cycloalkyl, C₃₋₇cycloalkylC₁₋₁₀alkyl, C₅₋₇cycloalkenyl, C₅₋₇ cycloalkenylC₁₋₁₀alkyl, (CR₁₀R₂₀)_nOR₆, (CR₁₀R₂₀)_nSH, (CR₁₀R₂₀)_nS(O)_mR₇, (CR₁₀R₂₀)_nNR₁₀S(O)₂R₇, (CR₁₀R₂₀)_nNR₄R₁₄, (CR₁₀R₂₀)_nCN, (CR₁₀R₂₀)_nS(O)₂NR₄R₁₄, (CR₁₀R₂₀)_nC(Z)R₆, (CR₁₀R₂₀)_nOC(Z)R₆,

$(CR_{10}R_{20})_nC(Z)OR_6$, $(CR_{10}R_{20})_nC(Z)NR_4R_{14}$, $(CR_{10}R_{20})_nNR_{10}C(Z)R_6$,
 $(CR_{10}R_{20})_nNR_{10}C(=NR_{10})NR_4R_{14}$, $(CR_{10}R_{20})_nOC(Z)NR_4R_{14}$,
 $(CR_{10}R_{20})_nNR_{10}C(Z)NR_4R_{14}$, or $(CR_{10}R_{20})_nNR_{10}C(Z)OR_7$.

Preferably, one or more of A₁ to A₃ is substituted with $(CR_{10}R_{20})_nOR_6$.

5 More preferably, R₆ is hydrogen.

A preferred C(A₁)(A₂)(A₃) grouping is CH(CH₂OH)₂, or C(CH₃)(CH₂OH)₂, X₁(CR₁₀R₂₀)_qCH(CH₂OH)₂, or X₁(CR₁₀R₂₀)_qC(CH₃)(CH₂OH)₂. X₁ is preferably oxygen or nitrogen.

10 As used herein, "optionally substituted" unless specifically defined shall mean such groups as halogen, such as fluorine, chlorine, bromine or iodine; hydroxy; hydroxy substituted C₁₋₁₀alkyl; C₁₋₁₀ alkoxy, such as methoxy or ethoxy; halosubstituted C₁₋₁₀ alkoxy; S(O)m alkyl, such as methyl thio, methylsulfinyl or methyl sulfonyl; -C(O); NR_{4'}R_{14'}, wherein R_{4'} and R_{14'} are each independently

15 hydrogen or C₁₋₄ alkyl, such as amino or mono or -disubstituted C₁₋₄ alkyl or wherein the R_{4'}R_{14'} can cyclize together with the nitrogen to which they are attached to form a 5 to 7 membered ring which optionally contains an additional heteroatom selected from O/N/S; C₁₋₁₀ alkyl, C₃₋₇cycloalkyl, or C₃₋₇cycloalkyl C₁₋₁₀ alkyl group, such as methyl, ethyl, propyl, isopropyl, t-butyl, etc. or cyclopropyl methyl; halosubstituted C₁₋₁₀ alkyl, such CF₂CF₂H, or CF₃; an optionally substituted aryl, 20 such as phenyl, or an optionally substituted arylalkyl, such as benzyl or phenethyl, wherein these aryl containing moieties may also be substituted one to two times by halogen; hydroxy; hydroxy substituted alkyl; C₁₋₁₀ alkoxy; S(O)malkyl; amino, mono & di-substituted C₁₋₄ alkyl amino, such as in the NR₄R₁₄ group; C₁₋₄ alkyl, or CF₃.

25 Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, ethane sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, 30 phenylacetic acid and mandelic acid.

In addition, pharmaceutically acceptable salts of compounds of Formula (I) may also be formed with a pharmaceutically acceptable cation, for instance, if a substituent group comprises a carboxy moiety. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, 35 ammonium and quaternary ammonium cations.

The term "halo" or "halogens" is used herein to mean the halogens, chloro, fluoro, bromo and iodo.

The term "C₁₋₁₀alkyl" or "alkyl" or "alkyl1-10" is used herein to mean both straight and branched chain radicals of 1 to 10 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl and the like.

5 The term "cycloalkyl" is used herein to mean cyclic radicals, preferably of 3 to 8 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.

10 The term "cycloalkenyl" is used herein to mean cyclic radicals, preferably of 5 to 8 carbons, which have at least one bond including but not limited to cyclopentenyl, cyclohexenyl, and the like.

15 The term "alkenyl" is used herein at all occurrences to mean straight or branched chain radical of 2-10 carbon atoms, unless the chain length is limited thereto, including, but not limited to ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-but enyl, 2-but enyl and the like.

20 The term "aryl" is used herein to mean phenyl and naphthyl.

25 The term "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") is used herein to mean a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited to, pyrrole, pyrazole, furan, pyran, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, pyridazine, pyrazine, uracil, oxadiazole, oxazole, isoxazole, oxathiadiazole, thiazole, isothiazole, thiadiazole, tetrazole, triazole, indazole, imidazole, or benzimidazole.

30 The term "heterocyclic" (on its own or in any combination, such as "heterocyclalkyl") is used herein to mean a saturated or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, S, or S(O)_m, and m is 0 or an integer having a value of 1 or 2; such as, but not limited to, the saturated or partially saturated versions of the heteroaryl moieties as defined above, such as tetrahydropyrrole, tetrahydropyran, tetrahydrofuran, tetrahydrothiophene (including oxidized versions of the sulfur moiety), pyrrolidine, piperidine, piperazine, morpholine, thiomorpholine (including oxidized versions of the sulfur moiety), or imidazolidine.

35 The term "aralkyl" or "heteroarylalkyl" or "heterocyclicalkyl" is used herein to mean C₁₋₄ alkyl as defined above attached to an aryl, heteroaryl or heterocyclic moiety as also defined herein unless otherwise indicate.

The term "sulfinyl" is used herein to mean the oxide S(O) of the corresponding sulfide, the term "thio" refers to the sulfide, and the term "sulfonyl" refers to the fully oxidized S (O)₂ moiety.

The term "aroyl" is used herein to mean C(O)Ar, wherein Ar is as phenyl, naphthyl, or aryl alkyl derivative such as defined above, such group include but are not limited to benzyl and phenethyl.

5 The term "alkanoyl" is used herein to mean C(O)C₁₋₁₀ alkyl wherein the alkyl is as defined above.

It is recognized that the compounds of the present invention may exist as stereoisomers, regioisomers, or diastereomers. These compounds may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these individual compounds, isomers, and mixtures thereof are included within 10 the scope of the present invention.

Exemplified compounds of the compounds of this invention include the racemates, or optically active forms of the compounds of the working examples herein, and pharmaceutically acceptable salts thereof.

15 METHODS OF MANUFACTURE

The compounds of Formula (I), (Ia), (II) and (IIa) may be obtained by applying synthetic procedures, described herein. The synthesis provided for is applicable to producing compounds of Formula (I), (Ia), (II) and (IIa) having a variety of different R₁, R₂, Y, X, and R₃ groups which are reacted, employing optional substituents 20 which are suitably protected, to achieve compatibility with the reactions outlined herein. Subsequent deprotection, in those cases, then affords compounds of the nature generally disclosed. While a particular formula with particular substituent groups is shown herein, the synthesis is applicable to all formulas and all substituent groups herein.

Once the nucleus has been established, further compounds of Formula (I), (Ia), 25 (II) and (IIa) may be prepared by applying standard techniques for functional group interconversion, well known in the art. For instance: C(O)NR₄R₁₄ from CO₂CH₃ by heating with HNR₄R₁₄ in CH₃OH with or without catalytic or stoichiometric metal cyanide or Aluminum trimethyl, e.g. NaCN; OC(O)R₃ from OH with e.g., ClC(O)R₆ 30 in bases such as triethylamine and pyridine; NR₁₀-C(S)NR₄R₁₄ from NHR₁₀ with an alkylisothiocyanate, or thiocyanic acid and ClC(S)NR₄R₁₄; NR₁₀C(O)OR₆ from NHR₁₀ with an alkyl or aryl chloroformate; NR₁₀C(O)NR₄H from NHR₁₀ by treatment with an isocyanate, e.g. R₄N=C=O; NR₁₀-C(O)R₆ from NHR₁₀ by treatment with Cl-C(O)R₆ in pyridine; C(=NR₁₀)NR₄R₁₄ from C(NR₄R₁₄)S with 35 H₃NR₁₀⁺OAc⁻ by heating in alcohol; C(NR₄R₁₄)SR₆ from C(S)NR₄R₁₄ with R₆-I in an inert solvent, e.g. acetone; NR₁₀SO₂R₇ from NHR₁₀ by treatment with ClSO₂R₇ by heating in bases such as pyridine; NR₁₀C(S)R₆ from NR₁₀C(O)R₆ by treatment with Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3,2,4-

dithiadiphosphetane-2,4-disulfide]; NR₁₀SO₂CF₃ from NHR₁₀ with triflic anhydride and base wherein R₃, R₆, R₁₀, R₄ and R₁₄ are as defined in Formula (I) herein.

Precursors of the groups R₁, R₂ and R₃, can be other R₁, R₂ and R₃, etc. groups that may be interconverted by applying standard techniques for functional group interconversion. For example wherein a moiety is a halo substituted C₁₋₁₀ alkyl can be converted to the corresponding C₁₋₁₀ alkylN₃ derivative by reacting with a suitable azide salt, and thereafter if desired can be reduced to the corresponding C₁₋₁₀alkylNH₂ compound, which in turn can be reacted with R₇S(0)₂X wherein X is halo (e.g., chloro) to yield the corresponding C₁₋₁₀alkylNHS(0)₂R₇ compound.

Alternatively wherein the moiety is a halo-substituted C₁₋₁₀-alkyl it can be reacted with an amine R₄R₁₄NH to yield the corresponding C₁₋₁₀-alkylNR₄R₁₄ compound, or can be reacted with an alkali metal salt of R₇SH to yield the corresponding C₁₋₁₀alkylSR₇ compound.

As noted above, it may be desirable during the synthesis of the compounds of this invention, to derivatize reactive functional groups in the molecule undergoing reaction so as to avoid unwanted side reactions. Functional groups such as hydroxy, amino, an acid groups typically are protected with suitable groups that can be readily removed when desired. Suitable common protecting groups for use with hydroxyl groups and nitrogen groups are well known in the art and described in many references, for instance, Protecting Groups in Organic Synthesis, Greene et al., John Wiley & Sons, New York, New York, (2nd edition, 1991 or the earlier 1981 version). Suitable examples of hydroxyl protecting groups include ether forming groups such as benzyl, and aryl groups such as *tert*-butoxycarbonyl (Boc), silyl ethers, such as *t*-butyldimethyl or *t*-butyldiphenyl, and alkyl ethers, such as methyl connected by an alkyl chain of variable link, (CR₁₀R₂₀)_n. Amino protecting groups may include benzyl, aryl such as acetyl and trialkylsilyl groups. Carboxylic acid groups are typically protected by conversion to an ester that can easily be hydrolyzed, for example, trichloethyl, *tert*-butyl, benzyl and the like.

Pharmaceutically acid addition salts of compounds of Formula (I), (Ia), (II) and (IIa) may be obtained in known manner, for example by treatment thereof with an appropriate amount of acid in the presence of a suitable solvent.

An illustration of the preparation of compounds of the present invention is shown in the scheme below. For purposes herein, the compounds in Schemes I and II are shown with an S-methyl, or S(O)₂-methyl group which is deemed representative of the S(O)m-Rg group, as described in the formulas below.

The starting material 1-Scheme I may be obtained from the commercially available 4,6-dihydroxy-2-methylmercaptopurimidine by known literature procedures,

such as those noted in Santilli et al., *J. Heterocycl. Chem.* (1971), 445-53, wherein POCl₃ and DMF are used.

The intermediate **2-Scheme I** was produced by two different routes. In the first route, coupling of dichloro aldehyde **1-Scheme I** with aryl amines in the presence of NaH in DMSO (Santilli et al., *J. Heterocycl. Chem.* (1971), 445-53) afforded the desired compound **2-Scheme I** along with imine **13-Scheme I**. The imine was converted to aldehyde **2-Scheme I** by treatment with aqueous HCl in THF.

Conversion of **1-Scheme I** to **2-Scheme I** may also be achieved using triethylamine and the desired amine in chloroform at room temperature for 10 minutes. The reaction was very effective for a range of alkyl amines (78-95 % yield). For aryl amines, elevated temperatures (reflux) and longer reaction time (24 hours) were necessary for reaction completion. Use of the base could be omitted when 3 or more equivalent of amine were used. Other suitable bases, include but are not limited to pyridine, diisopropyl ethylamine or pyrrolidine, which may also be used in an appropriate organic solvent, including but not limited to THF, diethyl ether or dioxane.

In the second route, the nitrile **9-Scheme I** was prepared in three steps from the aldehyde **1-Scheme I** (Santilli et al., *J. Heterocycl. Chem.* (1971), 445-53). Coupling of dichloro nitrile **9-Scheme I** with aryl amines in the presence of NaH in DMSO afforded the desired compound **10-Scheme I**. Other suitable bases such as pyridine, diisopropyl ethylamine, or sodium may also be used in an appropriate organic solvent such as THF, DMF or dioxane. Production and use of the nitrile **9-Scheme-I** may also be found in PCT/US01/06688, filed March 2, 2001, published as WO 01/64679 whose disclosure is incorporated herein by reference in its entirety.

The nitrile **10-Scheme I** was easily reduced with DIBAL in dichloromethane at room temperature (Boschelliat et al., *J. Med. Chem.* (1998), 4365-4377) to afford desired **2-Scheme I** along with the unsubstituted imine **13-Scheme I** (R=H). The latter was hydrolyzed to **2-Scheme I** *in situ* with HCl. Other reduction agents, such as lithium aluminum hydride, Raney Ni, or SnCl₂, may be utilized in an appropriate organic solvent such as THF, diethyl ether or dioxane to perform the conversion of **10-Scheme I** to **2-Scheme I**.

Aldehyde **2-Scheme I** was coupled to arylboronic acids under Suzuki coupling conditions, using a palladium catalyst, such as tetrakis(triphenylphosphine) palladium(0), to afford good to excellent yields of **3-Scheme I**. Alternatively, the bi-aryl coupling reaction of **2-Scheme I** may be performed using aryl or heteroaryl organozinc, organocupper, organotin, or other organometallic reagents known to afford bi-aryl cross-coupling products such as **3-Scheme I** [see for example Solberg, J.; Undheim, K. *Acta Chemica Scandinavica* **1989**, 62-68]. Displacement of the chlorine in **2-Scheme I** may also be achieved with nitrogen nucleophiles [for related

aminations see US patent 3,631,045 and 3,910,913], sulphur nucleophiles, [see Tumkevicius, S. *Liebigs Ann.* 1995, 1703-1705], oxygen nucleophiles, or alkyl nucleophiles.

3-Scheme I was then converted to pyridopyrimidinone 5-Scheme I by one of three procedures. The first procedure used the Wittig reaction, as modified by Horner-Emmons, converting 3-Scheme I to 4-Scheme I. In this reaction, the aldehyde 3-Scheme I was treated with a suitable phosphorus ylide, such as triethyl phosphonoacetate or methyl diethylphosphonoacetate, to give the olefin intermediate 4-Scheme I. The reaction was performed under reflux, in a suitable base, such as sodium hydride, sodium methoxide, or sodium hydroxide, and in a suitable organic solvent such as diethyl ether, dioxane or ethanol. The conversion of 3-Scheme I to 4-Scheme I may also be performed using the Peterson olefination reaction, or an aldol-based olefination reaction that utilizes acetic anhydride, malonic acid and its monoalkyl esters, or ethyl acetate.

Heating of 4-Scheme I in toluene at 220 °C in a sealed tube (Matyus et al. *Heterocycles* (1985), 2057-64), followed by solvent removal, afforded the desired product 5-Scheme I. This reaction may be run in the presence of a suitable base, such as DBU or diisopropylethyl amine, pyridine, lithium bi(trimethylsilyl)amide, or LDA and in an appropriate organic solvent such as an organic hydrocarbon, cresol, dioxane, DMF, pyridine, or xylene.

The second procedure used a Horner-Emmons reaction with Still modification (Still et al., *Tetrahedron Lett.* (1983), 4405-8; Jacobsen et al., *Tetrahedron* (1994), 4323-34) to produce a mixture of desired product 5-Scheme I and *trans* isomer 4-Scheme I. *Trans* isomer 4-Scheme I was isolated and converted to the desired product 5-Scheme I by heating to 220 °C in toluene in a sealed tube as described above.

The third procedure involved acetylation of 3-Scheme I, followed by the intramolecular aldol condensation, promoted by an acetylating agent (such as acetic anhydride, acetyl chloride, or a ketene) and a suitable base (such as pyridine, diisopropyl ethylamine, or pyrrolidine), to generate 5-Scheme I in a very good yield. The third procedure is optimal when R₃ is an optionally substituted aryl, or heteroaryl. When R₃ is an arylalkyl, or heteroarylalkyl substituent it is not clear that the reaction will form the key intermediate of Formula (VII), as shown below (3a-Scheme II), which may optionally be isolated, as shown in Scheme II below. Compounds of Formula (VII) are preferably not isolated but further reacted with a base or with heat to cyclize into 5-Scheme-I. The first and second procedures should be utilized for all other R₃ moieties.

Oxidation of the sulfide 5-Scheme I to the sulfone 6-Scheme I was performed using *meta*-chloroperoxybenzoic acid (mCPBA) in high yield and purity. Suitable oxidation methods for use herein include use of one or two equivalents of *meta*-chloroperoxybenzoic acid (mCPBA) or Oxone® to afford either the sulfoxides or sulfones. Oxidation of the sulfides to sulfoxides or sulfones can also be effected by OsO₄ and catalytic tertiary amine N-oxide, hydrogen peroxide, other peracids, oxygen, ozone, organic peroxides, potassium and zinc permanganate, potassium persulfate, and sodium hypochlorite.

Displacements of the sulfones 6-Scheme I to the final products 7-Scheme-I were usually done with an excess of amine in N-methylpyrrolidine (Barvian et al., *J. Med. Chem.* (2000), 4606-4616). A wide range of primary amines underwent this reaction with excellent yields. In some cases (in O-displacement or sulfonamide formation) an anion of the nucleophile was prepared with base (usually sodium hydride) in dimethylformamide and then added to the sulfone. Yields for these reactions were usually lower. Similarly related sulfones and sulfoxides of the compounds herein wherein X is SO-alkyl or SO₂-alkyl have been reported in the literature to be displaced by a wide variety of nucleophiles. Thus the analogs of the compounds herein wherein X is an alkyl sulfone or sulfoxide may be displaced by primary and secondary alkylamines without additional base catalysis, preferably in a polar aprotic solvent, such as but not limited to, N-methyl pyrrolidin-2-one (NMP), and at varying temperatures depending upon the nucleophilicity of the amine. For instance displacement of the sulfone of analogs of Formula (I) compounds with ethanolamine, in NMP, occurred in 30 min. at 65° C, while a more hindered amine such as tris(hydroxymethyl)-aminomethane may require elevated temperatures and extended reaction times (80° C over a 24 hour reaction time). The sulfone may also be displaced with a substituted arylamine, or heteroarylamine at elevated temperatures, sometimes requiring formation of the aryl or heteroarylamine anion with sodium hydride, or other suitable base, in DMSO. In addition, the sulfoxide analogs of Formula (I) compounds may be readily displaced with aluminum salts of aryl or heteroaryl amines as previously described in the patent literature (WO 99/32121). Likewise, sulfone and sulfoxide analogs of Formula (I) and (Ia) may be displaced with aryl or heteroaryl or alkyl thiols or alkyl or aryl or heteroaryl alcohols. For instance analogs of (I) containing sulfones as the X substituents may be displaced with sodium alkoxide in the alcohol, or alternatively reactive alkoxide or phenoxide nucleophiles may be generated from the alcohol or phenol with a suitable base such as sodium, NaH or sodium bistrimethylsilyl amide in a polar aprotic solvent such as DMSO, or run as a neat reaction. Similarly sulfones related to Formula (I) and (Ia), for instance, may be displaced with carbon nucleophiles such as aryl or alkyl Grignard reagents or related

organometallics such as organo lithium, zinc, tin or boron. These reactions may, in some cases, require transition metal catalysis such as with Pd or Ni catalysts.

Displacement of related 2-pyrimidine sulfones with cyanide, malonate anions, unactivated enolates, or heterocyclic C nucleophiles such as 1-methylimidazole anion,

- 5 by the generation of the anion with NaH or other suitable base in THF also has precedent (see for example, *Chem Pharm Bull.* **1987**, 4972-4976.). For example, analogs of Formula (I) and (Ia) compounds wherein X is an alkyl sulfone may be displaced with the anion of 1-methyl imidazole, generated by treatment of 1-methyl imidazole with n-butyl lithium in a solvent such as THF at temperatures of about -70°, 10 to afford the C-alkylated product substituted on the imidazole C-2.

For the purposes herein, compounds of Formulas (I), (Ia), (II) and (IIa) wherein X is R₂ or NHS(O)mR₂ may be obtained from compounds of **6-Scheme I** by

displacement of the sulfone using the appropriate "X" functionality as defined in Formula (I) and (Ia). To obtain compounds of Formulas (I), (Ia), (II) and (IIa) wherein 15 X is S(O)_mR₂ and R₂ is other than methyl, displacement of the sulfone on the corresponding compound **6-Scheme I** by thiol (R₂SH) and then followed by

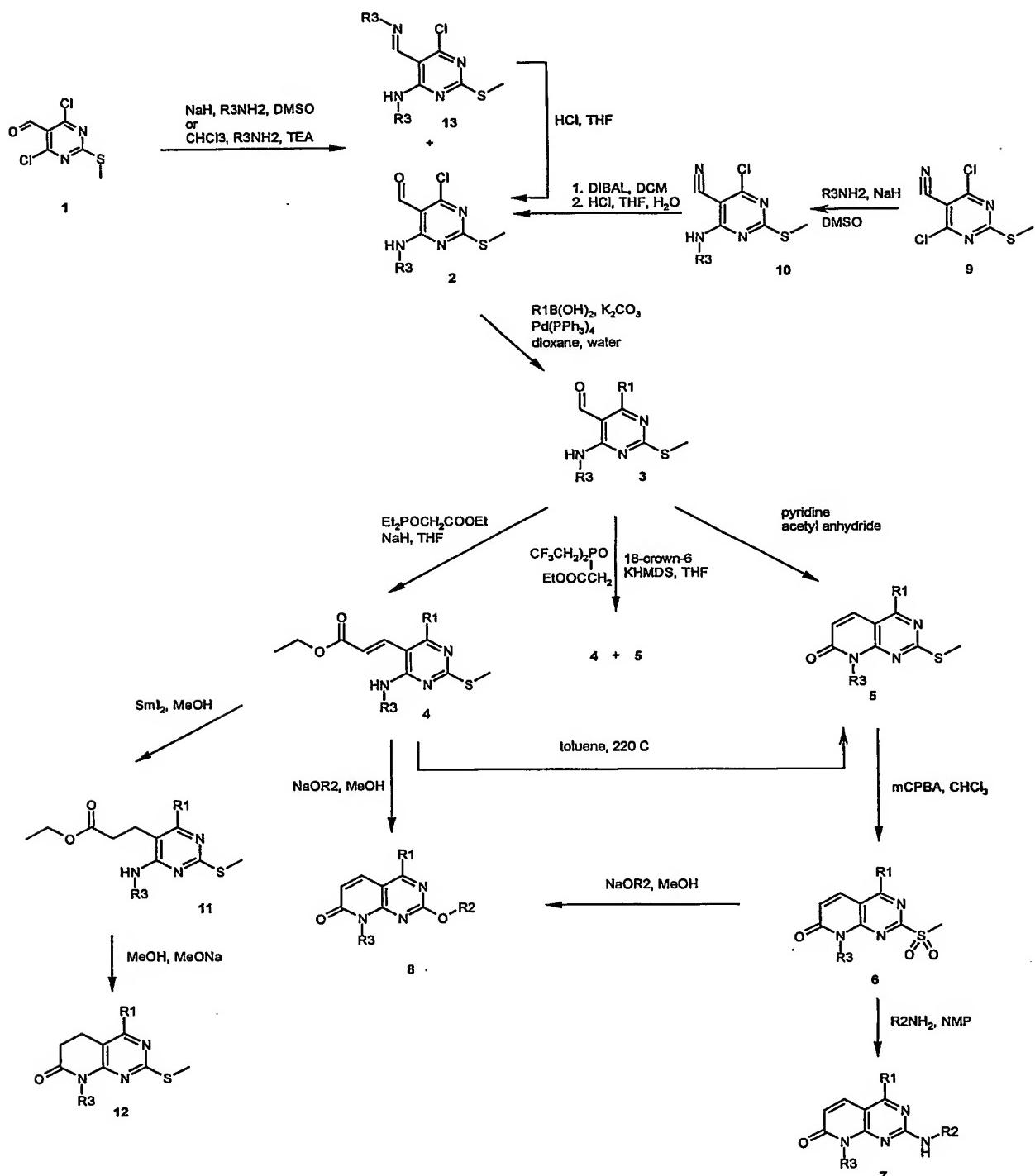
oxidation, if desired, with an appropriate oxidizing agent, such as MCPBA, or KMnO₄. Suitable oxidation methods for use herein include use of an oxidant such as one or two equivalents of *meta*-chloroperoxybenzoic acid or Oxone® to afford either 20 the sulfoxides or sulfones. Oxidation of the sulfides to sulfones may also be effected by OsO₄ and catalytic tertiary amine N-oxide. Other methods for sulfide oxidation include the use of hydrogen peroxide, other peracids, oxygen, ozone, organic peroxides, potassium and zinc permanganate, potassium persulfate, and sodium hypochlorite.

25 **8-Scheme I** can be also prepared by heating the *trans* ester **4-Scheme I** in alcohol in the presence of the corresponding sodium alkoxide. The yield of this reaction was very high for primary alcohols, but longer reaction times were required for secondary alcohols. Sodium alkoxides may be easily prepared from corresponding alcohol and base, such as sodium or sodium hydride.

30 Reduction of *trans* ester **4-Scheme I** with SmI₂ gives the reduced analogue **11-Scheme I**. This reduction can be also done in the presence of other reducing agents such as hydrogen gas, lithium in liquid ammonia, magnesium or sodium borohydride in the appropriate organic solvent such as THF, ethanol or diethyl ether.

Cyclization of the ester **11-Scheme I** can be done utilizing sodium methoxide 35 in methanol to give reduced analogue **12-Scheme I**. Other organic bases, such as sodium, sodium ethoxide or TEA can be used in an appropriate organic solvent such as methanol, ethanol or dioxane. The product **12-Scheme I** can be also obtained by

heating ester **11**-Scheme I to 150 °C in an appropriate organic solvent, such as toluene, xylene or isopropanol.



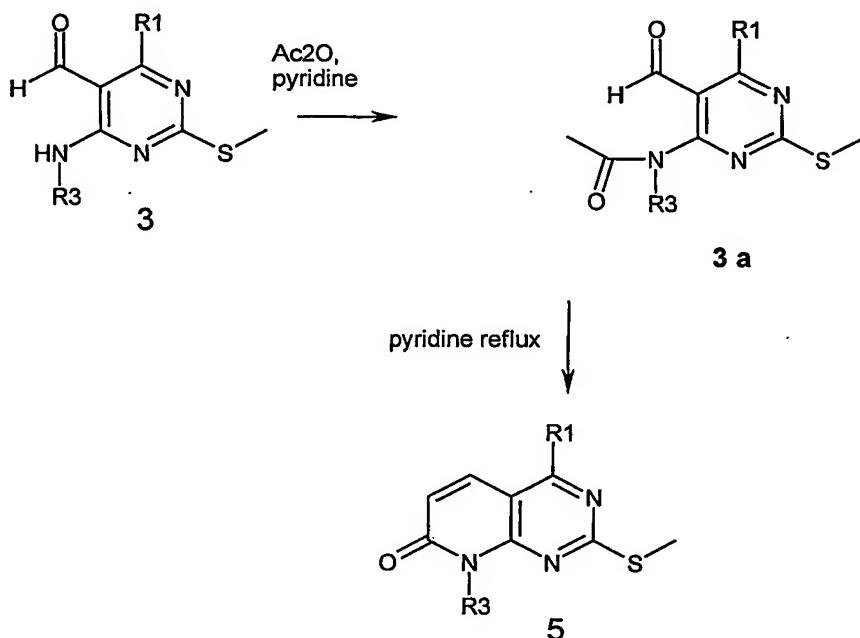
Scheme I

Additional procedures for producing similar intermediates to those herein, which the skilled artisan may find may be found in WO 99/41253, now US patent

6,200,977; US 6,153,619; US 6,268,310; US 5,468,751; US 5,474,996; and EP 1 040 831.

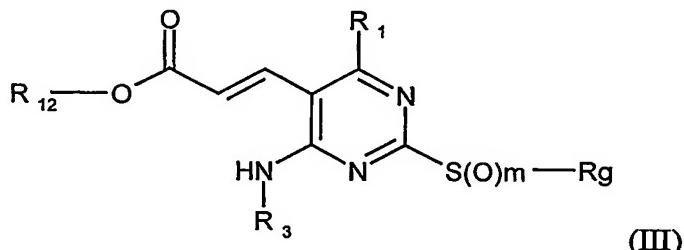
An illustration of an alternative preparation of compounds of Formula (VII) the present invention is shown in Scheme II below, and described above.

5



Scheme II

Another aspect of the present invention are novel intermediates of the formula
10 (III)



wherein

R₁ is an aryl or heteroaryl ring, which ring is optionally substituted;

R₃ is an C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl,

15 heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclylC₁₋₁₀ alkyl

moiety, which moieties are optionally substituted;

R₁₂ is a C₁₋₁₀ alkyl, aryl, heteroaryl, or arylalkyl;

m is 0 or an integer having a value of 1 or 2; and

Rg is a C₁₋₄ alkyl.

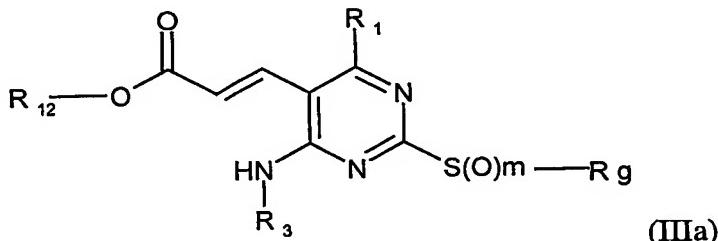
20

Preferably, R_g is a C₁₋₄ alkyl, and more preferably methyl.

Preferably, m is 0 or an integer having a value of 1 or 2. More preferably m is 0 or 2.

Preferably, R₁ is an aryl moiety, more preferably a phenyl ring, optionally substituted one or more times by halogen, C₁₋₄ alkyl, or halo-substituted-C₁₋₄ alkyl.
5 More preferably, the phenyl ring is substituted independently in the 2, 4, or 6-positions, or di-substituted in the 2,4- positions, such as 2-fluoro, 4-fluoro, 2,4-difluoro, 2,4,6-trifluoro, or 2-methyl-4-fluoro. More preferably, the phenyl ring is independently substituted in the 3 position, 2,3-disubstituted or 3,4-disubstituted, such
10 as by fluorine or chlorine.

Another aspect of the present invention are novel intermediates of the formula
(IIIa)



15 wherein

R₁ is the moiety YRa;

Y is C(R_b)(R_d), C(O), N(R_d), N(R_d)C(R_c)(R_d), oxygen, OC(R_c)(R_d), S(O)m, or S(O)_mC(R_c)(R_d);

R_a is an aryl or heteroaryl ring, which ring is optionally substituted;

20 R_b is hydrogen, C₁₋₂ alkyl, NR_c, hydroxy, thio, C₁₋₂ alkoxy, S(O)_mC₁₋₂ alkyl;

R_c is hydrogen or C₁₋₂ alkyl;

R_d is hydrogen or C₁₋₂ alkyl;

m is 0 or an integer having a value of 1 or 2; and

R₃ is an C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl,
25 heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclC₁₋₁₀ alkyl
moiety, which moieties are optionally substituted;

R₁₂ is a C₁₋₁₀ alkyl, aryl, heteroaryl, or arylalkyl;

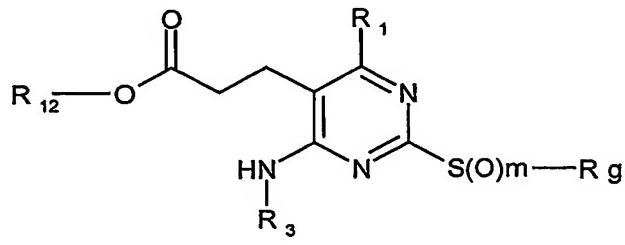
m is 0 or an integer having a value of 1 or 2; and

R_g is a C₁₋₄ alkyl.

30

The substituents of compounds of Formula (III) and (IIIa), and (IV) and (IVa) below follow those preferences of the final compounds of Formula (I) or (II) herein, respectively.

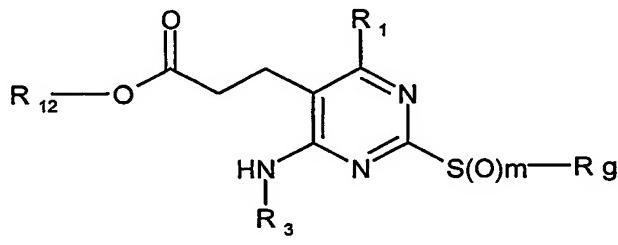
Another aspect of the present invention are the novel intermediates of the formula
 (IV)



(IV)

5 wherein R₁, R₃, R₁₂, m and R_g are as defined for Formula (III) above.

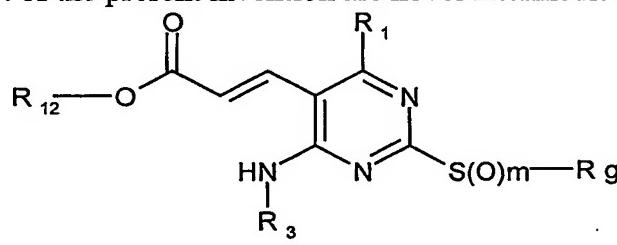
Another aspect of the present invention are the novel intermediates of the formula
 (IVa)



(IVa)

10 wherein R₁, R₃, R₁₂, m and R_g are as defined for Formula (IIIa) above.

Another aspect of the present invention are novel intermediates of the formula (IV)

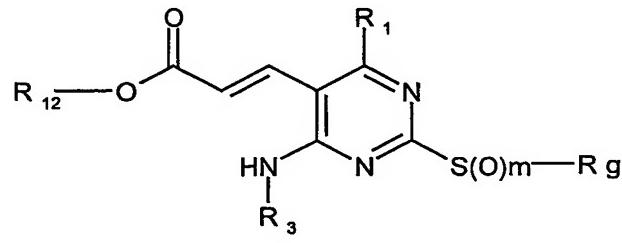


(V)

wherein R₁, R₃, R₁₂, m and R_g are as defined for Formula (III) above.

15

Another aspect of the present invention are novel intermediates of the formula
 (IVa)

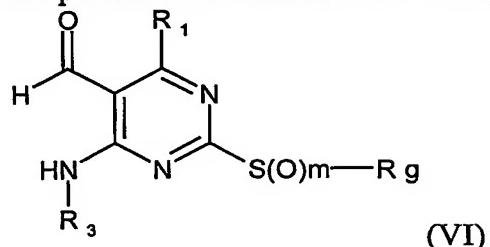


(Va)

wherein R₁, R₃, R₁₂, R_g and m are as defined for Formula (IIIa) above.

20

Another aspect of the present invention are novel intermediates of the formula



wherein

R_1 is a halogen, an optionally substituted aryl or an optionally substituted heteroaryl

5 ring;

R_3 is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclicC₁₋₁₀ alkyl moiety, which moieties are optionally substituted; provided that when R_3 is hydrogen, then R_1 is other than chlorine;

10 m is 0 or an integer having a value of 1 or 2; and

R_g is a C₁₋₄ alkyl.

Preferably, R_1 is a halogen, more preferably chlorine, or an aryl moiety, more preferably a phenyl ring, optionally substituted one or more times independently by halogen, C₁₋₄ alkyl, or halo-substituted-C₁₋₄ alkyl. More preferably, the phenyl ring is substituted in the 2, 4, or 6-positions, or di-substituted in the 2,4- positions, such as 2-fluoro, 4-fluoro, 2,4-difluoro, 2,4,6-trifluoro, or 2-methyl-4-fluoro. Alternatively, the phenyl ring is independently substituted in the 3 position, 2,3-disubstituted or 3,4-disubstituted, such as by fluorine or chlorine.

20

Preferably, R_3 is an optionally substituted C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, or aryl.

Preferably, the R_3 optional substituents are independently selected from C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇ cycloalkyl,

25 C₃₋₇cycloalkylC₁₋₁₀ alkyl, C₅₋₇ cycloalkenyl, C₅₋₇ cycloalkenyl C₁₋₁₀ alkyl, halogen, (CR₁₀R₂₀)_nOR₆, (CR₁₀R₂₀)_nSH, (CR₁₀R₂₀)_nS(O)_mR₇,

(CR₁₀R₂₀)_nNHS(O)₂R₇, (CR₁₀R₂₀)_nNR₄R₁₄, (CR₁₀R₂₀)_nCN, (CR₁₀R₂₀)_nS(O)₂NR₄R₁₄, (CR₁₀R₂₀)_nC(Z)R₆, (CR₁₀R₂₀)_nOC(Z)R₆, (CR₁₀R₂₀)_nC(Z)OR₆,

(CR₁₀R₂₀)_nC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z)R₆, (CR₁₀R₂₀)_nNR₁₀C(=NR₁₀)

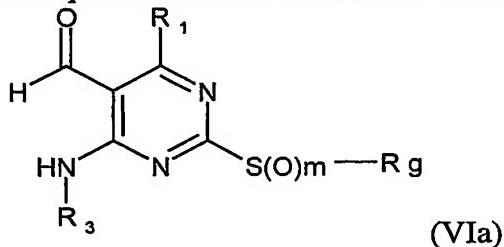
30 NR₄R₁₄, (CR₁₀R₂₀)_nOC(Z)NR₄R₁₄, (CR₁₀R₂₀)_nNR₁₀C(Z) NR₄R₁₄, or (CR₁₀R₂₀)_nNR₁₀C(Z)OR₇.

More preferably, the optional substituents are independently selected from halogen, alkyl, hydroxy, alkoxy, amino, or halosubstituted alkyl.

Exemplified compounds of Formula (VI) include, but are not limited to:

- 4-(2,4-difluoro-phenylamino)-6-(2,4,6-trifluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 5 4-(2,4-difluoro-phenyl)-6-(2,4-difluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 4-(2-fluorophenyl)-6-(2,4,6-trifluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 10 4-(4-fluorophenyl)-6-(2,4,6-trifluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 4-(2-fluorophenyl)-6-(2,4-difluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 15 4-(2,4-Difluoro-phenyl)-6-(4-fluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 4-(2,4-difluoro-phenyl)-6-(2,4-difluorophenylamino)-methylsulfanyl-pyrimidine-5-carbaldehyde;
- 15 4-(2-chloro-4-fluorophenyl)-6-(2,4-difluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde; or
- 4-(2,4-difluorophenyl)-6-(2,3-difluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde.

Another aspect of the present invention are novel intermediates of the formula



wherein

- 25 R₁ is YR_a;
- Y is C(R_b)(R_d), C(O), N(R_d), N(R_d)C(R_c)(R_d), oxygen, OC(R_c)(R_d), S(O)m, or S(O)_mC(R_c)(R_d);
- R_a is an aryl or heteroaryl ring, which ring is optionally substituted;
- R_b is hydrogen, C 1-2 alkyl, NR_c, hydroxy, thio, C 1-2 alkoxy, S(O)_mC₁₋₂ alkyl;
- 30 R_c is hydrogen or C 1-2 alkyl;
- R_d is hydrogen or C 1-2 alkyl;
- m is 0 or an integer having a value of 1 or 2; and

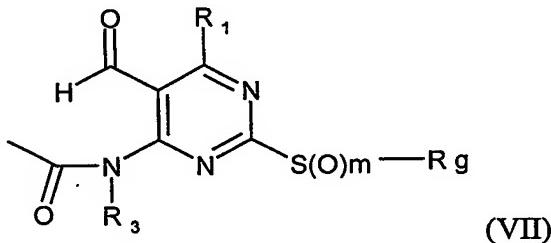
- R₃ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclicC₁₋₁₀ alkyl moiety, which moieties are optionally substituted;
- m is 0 or an integer having a value of 1 or 2; and
- 5 R_g is a C₁₋₄ alkyl.

Preferably, as noted above, the substituents of compounds of Formula (VI) and (VIa) follow those of the final compounds of Formula (I), and (II) herein.

- 10 Exemplified compounds of Formula (VI) include, but are not limited to, 4-(2-Chloro-phenylamino)-2-methylsulfanyl-6-phenoxy-pyrimidine-5-carbaldehyde.

Another aspect of this invention are novel intermediates of Formula (VII)

15

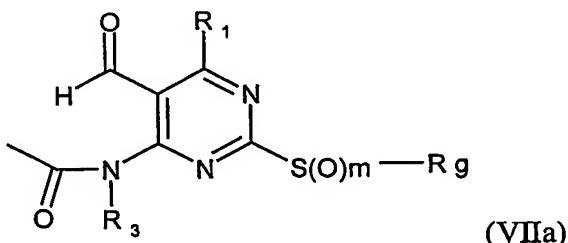


wherein

- R₁ is as defined above for Formula (I) compounds, and R₃, R_g, and m is an optionally substituted aryl or heteroaryl moiety, as defined for Formula (III) compounds.
- 20

Another aspect of this invention are novel intermediates of Formula (VIIa)

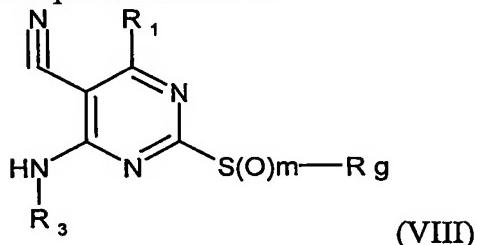
25



wherein

- R₁ is defined above for Formula (II) compounds, and R₃, R_g, and m is an optionally substituted aryl or heteroaryl moiety, as defined for Formula (IIIa) compounds.

Another aspect of the present invention are novel intermediates of the formula



wherein

- 5 R₁ is a halogen;
- R₃ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkylalkyl, aryl, arylC₁₋₁₀ alkyl, heteroaryl, heteroarylC₁₋₁₀ alkyl, heterocyclic, or a heterocyclicC₁₋₁₀ alkyl moiety, which moieties are optionally substituted; provided that when R₃ is hydrogen, then R₁ is other than chlorine;
- 10 m is 0 or an integer having a value of 1 or 2; and
- R_g is a C₁₋₄ alkyl.

Preferably R₁ is a halogen, more preferably chlorine.

- Suitably, the R₃ substituents are the same as those for compounds of Formulas (I) and (II) herein.

Representative compounds of Formula (I) and (Ia) are:

- 8-(2,4,6-trifluorophenyl)-4-(2,4-difluorophenyl)-2-(2-hydroxy-1-hydroxymethylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one;
- 20 4,8-Bis-(2,4-difluoro-phenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido-[2,3-d]-pyrimidin-7-one;
- 8-(2,4,6-trifluorophenyl)-4-(2-fluorophenyl)-2-(2-hydroxy-1-hydroxymethylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one;
- 8-(2,4,6-trifluorophenyl)-4-(4-fluorophenyl)-2-(2-hydroxy-1-hydroxymethylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one;
- 25 4-(2-fluoro-phenyl)-8-(2,4-difluoro-phenyl)-2-((S)-2-hydroxy-1-methyl-ethylamino)-8H-pyrido-[2,3-d]-pyrimidin-7-one;
- 4-(2,4-Difluoro-phenyl)-8-(4-fluoro-phenyl)-2-((S)-2-hydroxy-1-methyl-ethylamino)-8H-pyrido-[2,3-d]-pyrimidin-7-one;
- 30 4,8-Bis-(2,4-difluoro-phenyl)-2-(2-hydroxy-1-methyl-ethylamino)-8H-pyrido-[2,3-d]-pyrimidin-7-one;
- 8-(2,4-Difluorophenyl)-4-(2-chloro-4-fluorophenyl)-2-(2-hydroxy-1-hydroxymethylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one; or

8-(2,3-difluorophenyl)-4-(2,4-difluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one; or a pharmaceutically acceptable salt thereof.

5 **METHODS OF TREATMENT**

The compounds of Formula (I) and (Ia) or a pharmaceutically acceptable salt thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human, or other mammal, which is exacerbated or caused by excessive or unregulated cytokine production by such 10 mammal's cell, such as but not limited to monocytes and/or macrophages.

For purposes herein, compounds of Formula (I) and (Ia) will all be referred to as compounds of Formula (I) unless otherwise indicated.

Compounds of Formula (I) are capable of inhibiting proinflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF and are therefore of use in therapy. IL-1, 15 IL-6, IL-8 and TNF affect a wide variety of cells and tissues and these cytokines, as well as other leukocyte-derived cytokines, are important and critical inflammatory mediators of a wide variety of disease states and conditions. The inhibition of these pro-inflammatory cytokines is of benefit in controlling, reducing and alleviating many of these disease states.

20 Accordingly, the present invention provides a method of treating a cytokine-mediated disease which comprises administering an effective cytokine-interfering amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

Compounds of Formula (I) are capable of inhibiting inducible proinflammatory proteins, such as COX-2, also referred to by many other names such as prostaglandin 25 endoperoxide synthase-2 (PGHS-2) and are therefore of use in therapy. These proinflammatory lipid mediators of the cyclooxygenase (CO) pathway are produced by the inducible COX-2 enzyme. Regulation, therefore of COX-2 which is responsible for the these products derived from arachidonic acid, such as prostaglandins affect a wide variety of cells and tissues are important and critical 30 inflammatory mediators of a wide variety of disease states and conditions. Expression of COX-1 is not effected by compounds of Formula (I). This selective inhibition of COX-2 may alleviate or spare ulcerogenic liability associated with inhibition of COX-1 thereby inhibiting prostoglandins essential for cytoprotective effects. Thus 35 inhibition of these pro-inflammatory mediators is of benefit in controlling, reducing and alleviating many of these disease states. Most notably these inflammatory mediators, in particular prostaglandins, have been implicated in pain, such as in the sensitization of pain receptors, or edema. This aspect of pain management therefore includes treatment of neuromuscular pain, headache, cancer pain, and arthritis pain.

Compounds of Formula (I) or a pharmaceutically acceptable salt thereof, are of use in the prophylaxis or therapy in a human, or other mammal, by inhibition of the synthesis of the COX-2 enzyme.

Accordingly, the present invention provides a method of inhibiting the synthesis of COX-2 which comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. The present invention also provides for a method of prophylaxis treatment in a human, or other mammal, by inhibition of the synthesis of the COX-2 enzyme.

In particular, compounds of Formula (I) or a pharmaceutically acceptable salt thereof are of use in the prophylaxis or therapy of any disease state in a human, or other mammal, which is exacerbated by or caused by excessive or unregulated IL-1, IL-6, IL-8 or TNF production by such mammal's cell, such as, but not limited to, monocytes and/or macrophages.

Accordingly, in another aspect, this invention relates to a method of inhibiting the production of IL-1 in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

There are many disease states in which excessive or unregulated IL-1 production is implicated in exacerbating and/or causing the disease. These include rheumatoid arthritis, osteoarthritis, meningitis, ischemic and hemorrhagic stroke, neurotrauma/closed head injury, stroke, endotoxemia and/or toxic shock syndrome, other acute or chronic inflammatory disease states such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease, tuberculosis, atherosclerosis, muscle degeneration, multiple sclerosis, cachexia, bone resorption, psoriatic arthritis, Reiter's syndrome, gout, traumatic arthritis, rubella arthritis and acute synovitis. Recent evidence also links IL-1 activity to diabetes, pancreatic β cell diseases and Alzheimer's disease.

Use of a CSAID inhibitor compound for the treatment of CSBP mediated disease states, can include, but not be limited to neurodegenerative diseases, such as Alzheimer's disease (as noted above), Parkinson's disease and multiple sclerosis, etc..

In a further aspect, this invention relates to a method of inhibiting the production of TNF in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

Excessive or unregulated TNF production has been implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory

distress syndrome, chronic pulmonary inflammatory disease and chronic obstructive pulmonary disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, such as osteoporosis, cardiac, brain and renal reperfusion injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, such as influenza, brain infections including encephalitis (including HIV-induced forms), cerebral malaria, meningitis, ischemic and hemorrhagic stroke, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS, ARC (AIDS related complex), keloid formation, scar tissue formation, inflammatory bowel disease, Crohn's disease, ulcerative colitis and pyresis.

Compounds of Formula (I) are also useful in the treatment of viral infections, where such viruses are sensitive to upregulation by TNF or will elicit TNF production *in vivo*. The viruses contemplated for treatment herein are those that produce TNF as a result of infection, or those which are sensitive to inhibition, such as by decreased replication, directly or indirectly, by the TNF inhibiting-compounds of Formula (I). Such viruses include, but are not limited to HIV-1, HIV-2 and HIV-3, Cytomegalovirus (CMV), Influenza, adenovirus and the Herpes group of viruses, such as but not limited to, Herpes Zoster and Herpes Simplex. Accordingly, in a further aspect, this invention relates to a method of treating a mammal afflicted with a human immunodeficiency virus (HIV) which comprises administering to such mammal an effective TNF inhibiting amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

It is also recognized that both IL-6 and IL-8 are produced during rhinovirus (HRV) infections and contribute to the pathogenesis of common cold and exacerbation of asthma associated with HRV infection (Turner et al. (1998), Clin. Infec. Dis., Vol 25 26, p 840; Teren et al. (1997), Am J Respir Crit Care Med vol 155, p1362; Grunberg et al. (1997), Am J Respir Crit Care Med 156:609 and Zhu et al, J Clin Invest (1996), 97:421). It has also been demonstrated *in vitro* that infection of pulmonary epithelial cells with HRV results in production of IL-6 and IL-8 (Subauste et al., J. Clin. Invest. 1995, 96:549.) Epithelial cells represent the primary site of infection of HRV. Therefore another aspect of the present invention is a method of treatment to reduce inflammation associated with a rhinovirus infection, not necessarily a direct effect on virus itself.

Compounds of Formula (I) may also be used in association with the veterinary treatment of mammals, other than in humans, in need of inhibition of TNF production. TNF mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples of such viruses include, but are not limited to, lentivirus infections such as, equine infectious anaemia virus, caprine arthritis virus, visna virus, or maedi virus or

retrovirus infections, such as but not limited to feline immunodeficiency virus (FIV), bovine immunodeficiency virus, or canine immunodeficiency virus or other retroviral infections.

The compounds of Formula (I) may also be used topically in the treatment or prophylaxis of topical disease states mediated by or exacerbated by excessive cytokine production, such as by IL-1 or TNF respectively, such as inflamed joints, eczema, psoriasis and other inflammatory skin conditions such as sunburn; inflammatory eye conditions including conjunctivitis; pyrexia, pain and other conditions associated with inflammation. Periodontal disease has also been implemented in cytokine production, both topically and systemically. Hence use of compounds of Formula (I) to control the inflammation associated with cytokine production in such peroral diseases such as gingivitis and periodontitis is another aspect of the present invention.

Compounds of Formula (I) have also been shown to inhibit the production of IL-8 (Interleukin-8, NAP). Accordingly, in a further aspect, this invention relates to a method of inhibiting the production of IL-8 in a mammal in need thereof which comprises administering to said mammal an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

There are many disease states in which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. These diseases are characterized by massive neutrophil infiltration such as, psoriasis, inflammatory bowel disease, asthma, cardiac, brain and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis. All of these diseases are associated with increased IL-8 production which is responsible for the chemotaxis of neutrophils into the inflammatory site. In contrast to other inflammatory cytokines (IL-1, TNF, and IL-6), IL-8 has the unique property of promoting neutrophil chemotaxis and activation. Therefore, the inhibition of IL-8 production would lead to a direct reduction in the neutrophil infiltration.

The compounds of Formula (I) are administered in an amount sufficient to inhibit cytokine, in particular IL-1, IL-6, IL-8 or TNF, production such that it is regulated down to normal levels, or in some case to subnormal levels, so as to ameliorate or prevent the disease state. Abnormal levels of IL-1, IL-6, IL-8 or TNF, for instance in the context of the present invention, constitute: (i) levels of free (not cell bound) IL-1, IL-6, IL-8 or TNF greater than or equal to 1 picogram per ml; (ii) any cell associated IL-1, IL-6, IL-8 or TNF; or (iii) the presence of IL-1, IL-6, IL-8 or TNF mRNA above basal levels in cells or tissues in which IL-1, IL-6, IL-8 or TNF, respectively, is produced.

The discovery that the compounds of Formula (I) are inhibitors of cytokines, specifically IL-1, IL-6, IL-8 and TNF is based upon the effects of the compounds of

Formulas (I) on the production of the IL-1, IL-8 and TNF in *in vitro* assays which are described herein.

As used herein, the term "inhibiting the production of IL-1 (IL-6, IL-8 or TNF)" refers to:

- 5 a) a decrease of excessive *in vivo* levels of the cytokine (IL-1, IL-6, IL-8 or TNF) in a human to normal or sub-normal levels by inhibition of the *in* release of the cytokine by all cells, including but not limited to monocytes or macrophages;
- 10 b) a down regulation, at the genomic level, of excessive *in vivo* levels of the cytokine (IL-1, IL-6, IL-8 or TNF) in a human to normal or sub-normal levels;
- 10 c) a down regulation, by inhibition of the direct synthesis of the cytokine (IL-1, IL-6, IL-8 or TNF) as a posttranslational event; or
- 15 d) a down regulation, at the translational level, of excessive *in vivo* levels of the cytokine (IL-1, IL-6, IL-8 or TNF) in a human to normal or sub-normal levels.

As used herein, the term "TNF mediated disease or disease state" refers to any and all disease states in which TNF plays a role, either by production of TNF itself, or by TNF causing another monokine to be released, such as but not limited to IL-1, IL-6 or IL-8. A disease state in which, for instance, IL-1 is a major component, and whose production or action, is exacerbated or secreted in response to TNF, would therefore be considered a disease state mediated by TNF.

20 As used herein, the term "cytokine" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, inflammatory or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them. For instance, a monokine is generally referred to as being produced and secreted by a mononuclear cell, such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibroblasts, basophils, neutrophils, endothelial cells, brain astrocytes, bone marrow stromal cells, epidermal keratinocytes and B-lymphocytes. Lymphokines are generally referred to as being produced by lymphocyte cells. Examples of cytokines include, but are not limited to, Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF- α) and Tumor Necrosis Factor beta (TNF- β).

25 As used herein, the term "cytokine interfering" or "cytokine suppressive amount" refers to an effective amount of a compound of Formula (I) which will cause a decrease in the *in vivo* levels of the cytokine to normal or sub-normal levels, when given to a patient for the prophylaxis or treatment of a disease state which is exacerbated by, or caused by, excessive or unregulated cytokine production.

30 As used herein, the cytokine referred to in the phrase "inhibition of a cytokine, for use in the treatment of a HIV-infected human" is a cytokine which is implicated in

(a) the initiation and/or maintenance of T cell activation and/or activated T cell-mediated HIV gene expression and/or replication and/or (b) any cytokine-mediated disease associated problem such as cachexia or muscle degeneration.

As TNF- β (also known as lymphotoxin) has close structural homology with
5 TNF- α (also known as cachectin) and since each induces similar biologic responses and binds to the same cellular receptor, both TNF- α and TNF- β are inhibited by the compounds of the present invention and thus are herein referred to collectively as "TNF" unless specifically delineated otherwise.

A member of the MAP kinase family, alternatively termed CSBP, p38, or RK,
10 has been identified independently by several laboratories. Activation of this novel protein kinase via dual phosphorylation has been observed in different cell systems upon stimulation by a wide spectrum of stimuli, such as physicochemical stress and treatment with lipopolysaccharide or proinflammatory cytokines such as interleukin-1 and tumor necrosis factor. The cytokine biosynthesis inhibitors, of the present
15 invention, compounds of Formula (I) have been determined to be potent and selective inhibitors of CSBP/p38/RK kinase activity. These inhibitors are of aid in determining the signaling pathways involvement in inflammatory responses. In particular, for the first time a definitive signal transduction pathway can be prescribed to the action of lipopolysaccharide in cytokine production in macrophages. In addition to those
20 diseases already noted, treatment of stroke, neurotrauma, cardiac and renal reperfusion injury, congestive heart failure, coronary arterial bypass grafting (CABG) surgery, chronic renal failure, angiogenesis & related processes, such as cancer, thrombosis, glomerulonephritis, diabetes and pancreatic β cells, multiple sclerosis, muscle degeneration , eczema, psoriasis, sunburn, and conjunctivitis are also included.

25 The CSBP inhibitors were subsequently tested in a number of animal models for anti-inflammatory activity. Model systems were chosen that were relatively insensitive to cyclooxygenase inhibitors in order to reveal the unique activities of cytokine suppressive agents. The inhibitors exhibited significant activity in many such in vivo studies. Most notable are its effectiveness in the collagen-induced arthritis
30 model and inhibition of TNF production in the endotoxic shock model. In the latter study, the reduction in plasma level of TNF correlated with survival and protection from endotoxic shock related mortality. Also of great importance are the compounds effectiveness in inhibiting bone resorption in a rat fetal long bone organ culture system. Griswold et al., (1988) *Arthritis Rheum.* 31:1406-1412; Badger, et al., (1989)
35 *Circ. Shock* 27, 51-61; Votta et al., (1994) *in vitro. Bone* 15, 533-538; Lee et al., (1993). *B Ann. N. Y. Acad. Sci.* 696, 149-170.

Chronic diseases which have an inappropriate angiogenic component are various

ocular neovascularizations, such as diabetic retinopathy and macular degeneration. Other chronic diseases which have an excessive or increased proliferation of vasculature are tumor growth and metastasis, atherosclerosis, and certain arthritic conditions. Therefore CSBP kinase inhibitors will be of utility in the blocking of the 5 angiogenic component of these disease states.

The term "excessive or increased proliferation of vasculature inappropriate angiogenesis" as used herein includes, but is not limited to, diseases which are characterized by hemangiomas and ocular diseases.

10 The term "inappropriate angiogenesis" as used herein includes, but is not limited to, diseases which are characterized by vesicle proliferation with accompanying tissue proliferation, such as occurs in cancer, metastasis, arthritis and atherosclerosis.

15 Accordingly, the present invention provides a method of treating a CSBP kinase mediated disease in a mammal in need thereof, preferably a human, which comprises administering to said mammal, an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

20 In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of Formula (I) and a pharmaceutically acceptable carrier or diluent.

25 Compounds of Formula (I), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of Formula (I) may be administered in conventional dosage forms prepared by combining a compound of Formula (I) with standard pharmaceutical carriers according to conventional procedures. The compounds of Formula (I) may also be administered in conventional 30 dosages in combination with a known, second therapeutically active compound. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable character or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of 35 administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or 5 diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid 10 carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of Formula (I) may be administered topically, that is by non- 15 systemic administration. This includes the application of a compound of Formula (I) externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid 20 preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, for instance from 1% to 2% by weight of the formulation. 25 It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the formulation.

Lotions according to the present invention include those suitable for 30 application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid 35 formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural

origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a

- 5 polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a
10 suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100°C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the
15 container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

20 Compounds of Formula (I) may be administered parenterally, that is by intravenous, intramuscular, subcutaneous intranasal, intrarectal, intravaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred. Appropriate dosage forms for such administration may be prepared by conventional techniques. Compounds of Formula
25 (I) may also be administered by inhalation, that is by intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

For all methods of use disclosed herein for the compounds of Formula (I), the daily oral dosage regimen will preferably be from about 0.1 to about 80 mg/kg of total
30 body weight, preferably from about 0.2 to 30 mg/kg, more preferably from about 0.5 mg to 15mg. The daily parenteral dosage regimen about 0.1 to about 80 mg/kg of total body weight, preferably from about 0.2 to about 30 mg/kg, and more preferably from about 0.5 mg to 15mg/kg. The daily topical dosage regimen will preferably be from 0.1 mg to 150 mg, administered one to four, preferably two or three times daily. The
35 daily inhalation dosage regimen will preferably be from about 0.01 mg/kg to about 1 mg/kg per day. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of Formula (I) or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of

the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of Formula (I) or 5 a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The novel compounds of Formula (I) may also be used in association with the veterinary treatment of mammals, other than humans, in need of inhibition of 10 CSBP/p38 or cytokine inhibition or production. In particular, CSBP/p38 mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted herein in the Methods of Treatment section, but in particular viral infections. Examples of such viruses include, but are not limited to, lentivirus infections such as, equine infectious anaemia virus, caprine arthritis virus, visna virus, 15 or maedi virus or retrovirus infections, such as but not limited to feline immunodeficiency virus (FIV), bovine immunodeficiency virus, or canine immunodeficiency virus or other retroviral infections.

Another aspect of the present invention is a method of treating the common cold or respiratory viral infection caused by human rhinovirus (HRV), other 20 enteroviruses, coronavirus, influenza virus, parainfluenza virus, respiratory syncytial virus, or adenovirus in a human in need thereof which method comprises administering to said human an effective amount of a CBSP/p38 inhibitor.

Another aspect of the present invention is a method of treating, including prophylaxis of influenza induced pneumonia in a human in need thereof which method 25 comprises administering to said human an effective amount of a CBSP/p38 inhibitor.

The present invention also relates to the use of the CSBP/p38 kinase inhibitor for the treatment, including prophylaxis, of inflammation associated with a viral infection of a human rhinovirus (HRV), other enteroviruses, coronavirus, influenza virus, parainfluenza virus, respiratory syncytial virus, or adenovirus.

In particular, the present invention is directed to the treatment of a viral 30 infection in a human, which is caused by the human rhinovirus (HRV), other enterovirus, coronavirus, influenza virus, parainfluenza virus, respiratory syncytial virus, or an adenovirus. In particular the invention is directed to respiratory viral infections that exacerbate asthma (induced by such infections), chronic bronchitis, 35 chronic obstructive pulmonary disease, otitis media, and sinusitis. While inhibiting IL-8 or other cytokines may be beneficial in treating a rhinovirus may be known, the use of an inhibitor of the p38 kinase for treating HRV or other respiratory viral infections causing the common cold is believed novel. It should be noted that the

respiratory viral infection treated herein may also be associated with a secondary bacterial infection, such as otitis media, sinusitis, or pneumonia.

For use herein treatment may include prophylaxis for use in a treatment group susceptible to such infections. It may also include reducing the symptoms of, ameliorating the symptoms of, reducing the severity of, reducing the incidence of, or any other change in the condition of the patient, which improves the therapeutic outcome.

It should be noted that the treatment herein is not directed to the elimination or treatment of the viral organism itself but is directed to treatment of the respiratory viral infection that exacerbates other diseases or symptoms of disease, such as asthma (induced by such infections), chronic bronchitis, chronic obstructive pulmonary disease, otitis media, and sinusitis.

A preferred virus for treatment herein is the human rhinovirus infection (HRV) or respiratory syncytial virus (RSV).

15

The invention will now be described by reference to the following biological examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

BIOLOGICAL EXAMPLES

20

The cytokine-inhibiting effects of compounds of the present invention may be determined by the following *in vitro* assays: Assays for Interleukin - 1 (IL-1), Interleukin -8 (IL-8), and Tumour Necrosis Factor (TNF) are well known in the art, and may be found in a number of publications, and patents. Representative suitable assays for use herein are described in Adams et al., US 5,593,992, whose disclosure is incorporated by reference in its entirety.

25

Interleukin - 1 (IL-1)

Human peripheral blood monocytes are isolated and purified from either fresh blood preparations from volunteer donors, or from blood bank buffy coats, according to the procedure of Colotta *et al*, J Immunol, 132, 936 (1984). These monocytes (1×10^6) are plated in 24-well plates at a concentration of 1-2 million/ml per well. The cells are allowed to adhere for 2 hours, after which time non-adherent cells are removed by gentle washing. Test compounds are then added to the cells for 1h before the addition of lipopolysaccharide (50 ng/ml), and the cultures are incubated at 37°C for an additional 24h. At the end of this period, culture supernatants are removed and clarified of cells and all debris. Culture supernatants are then immediately assayed for IL-1 biological activity, either by the method of Simon *et al.*, J. Immunol. Methods, 84, 85, (1985) (based on ability of IL-1 to stimulate a Interleukin 2 producing cell line

(EL-4) to secrete IL-2, in concert with A23187 ionophore) or the method of Lee *et al.*, J. ImmunoTherapy, 6 (1), 1-12 (1990) (ELISA assay).

In vivo TNF assay:

- 5 (1) Griswold *et al.*, Drugs Under Exp. and Clinical Res.,XIX (6), 243-248
(1993); or
(2) Boehm, *et al.*, *Journal Of Medicinal Chemistry* 39, 3929-3937 (1996)
whose disclosures
are incorporated by reference herein in their entirety.

10

LPS-induced TNF α Production in Mice and Rats

In order to evaluate in vivo inhibition of LPS-induced TNF α production in rodents, both mice and rats are injected with LPS.

Mouse Method

15 Male Balb/c mice from Charles River Laboratories are pretreated (30 minutes) with compound or vehicle. After the 30 min. pretreat time, the mice are given LPS (lipopolysaccharide from Esherichia coli Serotype 055-85, Sigma Chemical Co., St Louis, MO) 25 ug/mouse in 25 ul phosphate buffered saline (pH 7.0) intraperitoneally. Two hours later the mice are killed by CO₂ inhalation and blood samples are collected
20 by exsanguination into heparinized blood collection tubes and stored on ice. The blood samples are centrifuged and the plasma collected and stored at -20°C until assayed for TNF α by ELISA.

Rat Method

25 Male Lewis rats from Charles River Laboratories are pretreated at various times with compound or vehicle. After a determined pretreat time, the rats are given LPS (lipopolysaccharide from Esherichia coli Serotype 055-85, Sigma Chemical Co., St Louis, MO) 3.0 mg/kg intraperitoneally. The rats are killed by CO₂ inhalation and heparinized whole blood is collected from each rat by cardiac puncture 90 minutes
30 after the LPS injection. The blood samples are centrifuged and the plasma collected for analysis by ELISA for TNF α levels.

ELISA Method

TNF α levels were measured using a sandwich ELISA, as described in Olivera
35 et al., Circ. Shock, 37, 301-306, (1992), whose disclosure is incorporated by reference in its entirety herein, using a hamster monoclonal antimurine TNF α (Genzyme, Boston, MA) as the capture antibody and a polyclonal rabbit antimurine TNF α (Genzyme) as the second antibody. For detection, a peroxidase-conjugated goat

antirabbit antibody (Pierce, Rockford, IL) was added, followed by a substrate for peroxidase (1 mg/ml orthophenylenediamine with 1% urea peroxide). TNF α levels in the plasma samples from each animal were calculated from a standard curve generated with recombinant murine TNF α (Genzyme).

5

LPS-Stimulated Cytokine Production in Human Whole Blood

Assay: Test compound concentrations were prepared at 10 X concentrations and LPS prepared at 1 μ g/ml (final conc. of 50 ng/ml LPS) and added in 50 μ L volumes to 1.5 mL eppendorf tubes. Heparinized human whole blood was obtained from healthy volunteers and was dispensed into eppendorf tubes containing compounds and LPS in 0.4 mL volumes and the tubes incubated at 37 C. Following a 4 hour incubation, the tubes were centrifuged at 5000 rpm for 5 minutes in a TOMY microfuge, plasma was withdrawn and frozen at -80 C.

10

Cytokine measurement: IL-1 and/or TNF were quantified using a standardized ELISA technology. An in-house ELISA kit was used to detect human IL-1 and TNF. Concentrations of IL-1 or TNF were determined from standard curves of the appropriate cytokine and IC₅₀ values for test compound (concentration that inhibited 50% of LPS-stimulated cytokine production) were calculated by linear regression analysis.

15

CSBP/p38 Kinase Assay:

This assay measures the CSBP/p38-catalyzed transfer of ³²P from [α -³²P]ATP to threonine residue in an epidermal growth factor receptor (EGFR)-derived peptide (T669) with the following sequence: KRELVEPLTPSGEAPNQALLR (residues 661-681). (See Gallagher *et al.*, "Regulation of Stress Induced Cytokine Production by Pyridinyl Imidazoles: Inhibition of CSBP Kinase", BioOrganic & Medicinal Chemistry, 1997, 5, 49-64).

20

Reactions were carried in round bottom 96 well plate (from Corning) in a 30 ml volume. Reactions contained (in final concentration): 25 mM Hepes, pH 7.5; 8 mM MgCl₂; 0.17 mM ATP (the Km[ATP] of p38 (see Lee *et al.*, Nature 300, n72 pg. 639-746 (Dec. 1994)); 2.5 uCi of [γ -³²P]ATP; 0.2 mM sodium orthovanadate; 1 mM DTT; 0.1% BSA; 10% glycerol; 0.67 mM T669 peptide; and 2-4 nM of yeast-expressed, activated and purified p38. Reactions were initiated by the addition of [γ -³²P]Mg/ATP, and incubated for 25 min. at 37 °C. Inhibitors (dissolved in DMSO) were incubated with the reaction mixture on ice for 30 minutes prior to adding the 32P-ATP. Final DMSO concentration was 0.16%. Reactions were terminated by adding 10 μ l of 0.3 M phosphoric acid, and phosphorylated peptide was isolated from the reactions by capturing it on p81 phosphocellulose filters. Filters were washed with

75 mM phosphoric acids, and incorporated ^{32}P was quantified using beta scintillation counter. Under these conditions, the specific activity of p38 was 400-450 pmol/pmol enzyme, and the activity was linear for up to 2 hours of incubation. The kinase activity values were obtained after subtracting values generated in the absence of substrate which were 10-15% of total values.

5 Representative final compounds of Formula (I) and (Ia) which have been tested, Examples 1 to 3, have all demonstrated positive inhibitory activity in this binding assay, having an IC₅₀ of < 10uM.

10 **TNF- α in Traumatic Brain Injury Assay**

This assay provides for examination of the expression of tumor necrosis factor mRNA in specific brain regions which follow experimentally induced lateral fluid-percussion traumatic brain injury (TBI) in rats. Since TNF- α is able to induce nerve growth factor (NGF) and stimulate the release of other cytokines from activated 15 astrocytes, this post-traumatic alteration in gene expression of TNF- α plays an important role in both the acute and regenerative response to CNS trauma. A suitable assay may be found in WO 97/35856 whose disclosure is incorporated herein by reference.

20 **CNS Injury model for IL- β mRNA**

This assay characterizes the regional expression of interleukin-1 β (IL-1 β) mRNA in specific brain regions following experimental lateral fluid-percussion traumatic brain injury (TBI) in rats. Results from these assays indicate that following TBI, the temporal expression of IL-1 β mRNA is regionally stimulated in specific brain 25 regions. These regional changes in cytokines, such as IL-1 β play a role in the post-traumatic pathologic or regenerative sequelae of brain injury. A suitable assay may be found in WO 97/35856 whose disclosure is incorporated herein by reference.

Angiogenesis Assay:

30 Described in WO 97/32583, whose disclosure is incorporated herein by reference, is an assay for determination of inflammatory angiogenesis which may be used to show that cytokine inhibition will stop the tissue destruction of excessive or inappropriate proliferation of blood vessels.

35 **Rhinovirus/Influenza Assay:**

Cell lines, rhinovirus serotype 39, and influenza virus A/PR/8/34 were purchased from American Type Culture Collection (ATCC). BEAS-2B cells were cultured according to instructions provided by ATCC using BEGM (bronchial

epithelial growth media) purchased from Clonetics Corp. HEA cell cultures, used for detection and titration of virus, were maintained in Eagle's minimum essential media containing 10% fetal calf serum, 2mM l-glutamine, and 10 mM HEPES buffer (MEM).

5 A modification of the method reported by Subauste et al., *Supra*, for in vitro infection of human bronchial epithelial cells with rhinovirus was used in these studies. BEAS-2B cells (2×10^5 /well) were cultured in collagen-coated wells for 24 hours prior to infection with rhinovirus. Rhinovirus serotype 39 was added to cell cultures for one hour incubation at 34°C after which inoculum was replaced with fresh media and
10 cultures were incubated for an additional 72 hours at 34°C. Supernatants collected at 72 hours post-infection were assayed for cytokine protein concentration by ELISA using commercially available kits (R&D Systems). Virus yield was also determined from culture supernatants using a microtitration assay in HEA cell cultures (*Subauste et al., supra 1995*). In cultures treated with p38 kinase inhibitors, drug was added 30
15 minutes prior to infection. Stocks of compounds were prepared in DMSO (10 mM drug) and stored at -20°C.

For detection of p38 kinase, cultures were incubated in basal media without growth factors and additives to reduce endogenous levels of activated p38 kinase. Cells were harvested at various timepoints after addition of rhinovirus. Detection of
20 tyrosine phosphorylated p38 kinase by immunoblot was analyzed by a commercially available kit and was performed according to the manufacturer's instructions (PhosphoPlus p38 MAPK Antibody Kit: New England BioLabs Inc.).

In some experiments, BEAS-2B cells were infected with influenza virus (strain A/PR/8/34) in place of rhinovirus. Culture supernatant was harvested 48 and 72 hour
25 post-infection and tested by ELISA for cytokine as described above.

Cells and Virus: Influenza A/PR/8/34 sub type H1N1 (VR-95 American Type Culture Collection, Rockville, MD) was grown in the allantoic cavity of 10 day old chicken eggs. Following incubation at 37°C, and refrigeration for 2 1/2 hours at 4°C, allantoic fluid was harvested, pooled, and centrifuged (1,000 rcf; 15 min; 4°C) to remove cells.
30 Supernatent was aliquoted and stored at -70°C. The titer of the stock culture of virus was 1.0×10^{10} Tissue Culture Infective Dose/ml (TCID₅₀)

Inoculation procedure: Four-six week old female Balb/cAnNcr1Br mice were obtained from Charles River, Raleigh, NC. Animals were infected intranasally. Mice were anesthetized by intraperitoneal injection of Ketamine (40mg/kg; Fort Dodge
35 Labs, Fort Dodge, Ia) and Xylazine (5 mg/kg; Miles, Shawnee Mission, Ks) and then inoculated with 100 TCID₅₀ of PR8 diluted in PBS in 20 ul. Animals were observed daily for signs of infection. All animal studies were approved by SmithKline Beecham Pharmaceuticals Institutional Animal Care and Use Committee.

Virus titration: At various times post infection, animals were sacrificed and lungs were aseptically harvested. Tissues were homogenized, in vials containing 1 micron glass beads (Biospec Products, Bartlesville, OK) and 1 ml. of Eagles minimal essential medium. Cell debris was cleared by centrifugation at 1,000 rcf for 15 minutes at 4°C, and supernatants were serially diluted on Madin-Darby canine kidney (MDCK) cells. After 5 days of incubation at 37°C (5% CO₂), 50 µl of 0.5% chick red blood cells were added per well, and agglutination was read after 1 hour at room temperature. The virus titer is expressed as 50% tissue culture infective dose (TCID₅₀) calculated by logistic regression.

ELISA: Cytokine levels were measured by quantitative ELISA using commercially available kits. Ear samples were homogenized using a tissue minser in PBS. Cell debris was cleared by centrifugation at 14,000 rpm for 5 minutes. The cytokine concentrations and thresholds were determined as described by the manufacturer; IL-6, IFN-γ, and KC (R&D Systems, Minneapolis, MN).

Myeloperoxidase Assay: Myeloperoxidase (MPO) activity was determined kinetically as described by Bradley et al. (1982). Briefly, rabbit cornea were homogenized in Hexadecyl Trimethyl-Ammonium Bromide (HTAB) (Sigma Chemical Co. St. Louis, Mo) which was dissolved in 0.5 m Potassium phosphate buffer (J.T. Baker Scientific, Phillipsburg, NJ). Following homogenization, the samples were subjected to freeze-thaw-sonication (Cole-Parmer 8853, Cole-Parmer, Vernon Hills, IL) 3 times. Suspensions were then cleared by centrifugation at 12,500 x g for 15 minutes at 4°C. MPO enzymatic activity was determined by colormetric change in absorbance during a reaction of O-Dianisidine dihydrochloride (ODI) 0.175 mg/ml (Sigma Chemical Co. St. Louis, Mo) with .0002% Hydrogen peroxide (Sigma Chemical Co. St. Louis, Mo). Measurements were performed by using a Beckman Du 640 Spectrophotometer (Fullerton, Ca.) fitted with a temperature control device. 50 µl of material to be assayed was added to 950 µl of ODI and change in absorbance was measured at a wave length of 460 nm for 2 minutes at 25°C.

Whole Body Plethysmography: Influenza virus infected mice were placed into a whole body plethysmograph box with an internal volume of approximately 350-ml. A bias airflow of one l/min was applied to the box and flow changes were measured and recorded with a Buxco XA data acquisition and respiratory analysis system (Buxco Electronics, Sharon, CT). Animals were allowed to acclimate to the plethysmograph box for 2 min. before airflow data was recorded. Airway measurements were calculated as Penh (enhanced pause). Penh has previously been shown as an index of airway obstruction and correlates with increased intrapleural pressure. The algorithm for Penh calculation is as follows: Penh = [(expiratory time /

relaxation time)-1] x (peak expiratory flow / peak inspiratory flow) where relaxation time is the amount of time required for 70% of the tidal volume to be expired.

Determination of arterial oxygen saturation. A Nonin veterinary hand held pulse oximeter 8500V with lingual sensor (Nonin Medical, Inc., Plymouth MN) was used to determine daily arterial oxygen saturation %SpO₂ as described (Sidwell et al. 1992 Antimicrobial Agents and Chemotherapy 36:473-476).

Additional data and assay modifications may be found in PCT/US00/25386, (WO 01/19322) filed 15 September 2000, whose disclosure is incorporated herein by reference in its entirety.

10

Fluorescence anisotropy kinase binding assay

The CSBP kinase enzyme, a fluorescent ligand and a variable concentration of the test compound are incubated together to reach thermodynamic equilibrium under conditions such that in the absence of test compound the fluorescent ligand is significantly (>50%) enzyme bound and in the presence of a sufficient concentration (>10x K_i) of a potent inhibitor the anisotropy of the unbound fluorescent ligand is measurably different from the bound value.

The concentration of kinase enzyme should preferably be $\geq 1 \times K_f$. The concentration of fluorescent ligand required will depend on the instrumentation used, and the fluorescent and physicochemical properties. The concentration used must be lower than the concentration of kinase enzyme, and preferably less than half the kinase enzyme concentration.

25

A typical protocol is:

All components dissolved in Buffer of final composition 62.5 mM HEPES, pH 7.5, 1.25 mM CHAPS, 1.25 mM DTT, 12.5 mM MgCl₂ 3.3% DMSO.

p38 Enzyme concentration: 12 nM

Fluorescent ligand concentration: 5 nM

30

Test compound concentration: 0.1 nM - 100 uM

Components incubated in 30 ul final volume in NUNC 384 well black microtitre plate until equilibrium reached (5-30 mins)

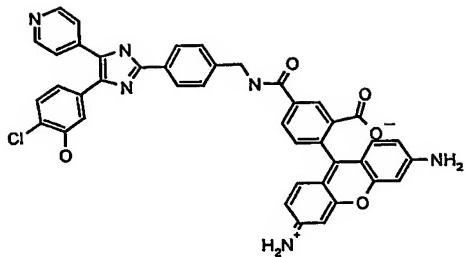
Fluorescence anisotropy read in L JL Acquest.

Definitions: K_i = dissociation constant for inhibitor binding

35

K_f = dissociation constant for fluorescent ligand binding

The fluorescent ligand is the following compound:



which is derived from 5-[2-(4-aminomethylphenyl)-5-pyridin-4-yl-1H-imidazol-4-yl]-2-chlorophenol and rhodamine green.

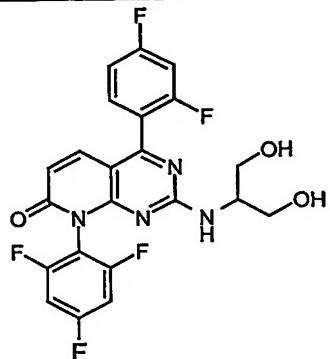
Representative final compounds of Formula (I) and (Ia) which have been
5 tested, Examples 1 to 4, 6, 8 and 9, have all demonstrated positive inhibitory activity
in this binding assay, having an IC₅₀ of < 1 uM.

SYNTHETIC EXAMPLES

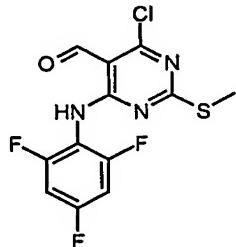
The invention will now be described by reference to the following examples
10 which are merely illustrative and are not to be construed as a limitation of the scope
of the present invention. All temperatures are given in degrees centigrade, all
solvents are highest available purity and all reactions run under anhydrous conditions
in an Argon (Ar) atmosphere where necessary.

Mass spectra were run on an open access LC-MS system using electrospray
15 ionization. LC conditions: 4.5% to 90% CH₃CN (0.02% TFA) in 3.2 min with a 0.4
min hold and 1.4 min re-equilibration; detection by MS, UV at 214 nm, and a light
scattering detector (ELS). Column: 1 X 40 mm Aquasil (C18) ¹H-NMR (hereinafter
"NMR") spectra were recorded at 400 MHz using a Bruker AM 400 spectrometer or
a Bruker AVANCE 400. Multiplicities indicated are: s=singlet, d=doublet, t=triplet,
20 q=quartet, m=multiplet and br indicates a broad signal. For preparative (prep) hplc;
ca 50 mg of the final products were injected in 500 uL of DMSO onto a 50 X 20
mm I. D. YMC CombiPrep ODS-A column at 20 mL/min with a 10 min gradient
from 10% CH₃CN (0.1% TFA) to 90% CH₃CN (0.1% TFA) in H₂O (0.1% TFA) and
a 2 min hold (unless otherwise stated). Flash chromatography was run over Merck
25 Silica gel 60 (230 - 400 mesh) in solvent mixtures containing varying relative
concentrations of dichloromethane and methanol, or EtOAc, and hexane, unless
otherwise stated. Chromatotron chromatography as has been previously described
(Desai, HK; Joshi, BS; Panu, AM; Pelletier, SW *J. Chromatogr.* **1985** 223-227.) was
run on chromatotron plates available from Analtech, Wilmington DE, USA.

30 satd = saturated; aq = aqueous; NMP = 1-methyl-2-pyrrolidinone; soln =
solution; other abbreviations are as described in the ACS Style Guide (American
Chemical Society, Washington, DC, 1986).

Example 1**8-(2,4,6-Trifluorophenyl)-4-(2,4-di-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one**

- 5 a) 4-Chloro-6-(2,4,6-tri-fluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde

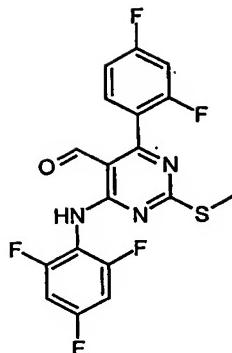


A solution of 4,6-dichloro-2-methylsulfanyl-pyrimidine-5-carbaldehyde [Santilli, et al., *J. Heterocycl. Chem.* 1971, 8, 445-45] (1.0 gram (hereinafter "g"), 4.48 millimoles (hereinafter "mmol")) in CHCl₃ (20 milliliters (hereinafter "mL")) was added 2,4,6-tri-fluoroaniline (0.735 g, 5 mmol) followed by Et₃N (0.94 mL, 6.72 mmol, 1.5 equivalents (hereinafter "eq")). The reaction mixture turned yellow and was heated at 50°C for 8 hours (hereinafter "h"), 1 M aq Na₂CO₃ solution (50 mL) was added and the layers were separated. The organic layer was washed with 50 ml sat'd. aqueous (hereinafter "satd aq") NaCl solution, dried through anhydrous MgSO₄ and evaporated. The crude product was purified by flash chromatography (Hex/AcOEt = 95/5) to afford 1.3 g (87 %) of pure 4-chloro-6-(2,4,6-trifluoro-phenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde.

LC MS (m/e) = 334.0 (MH⁺).

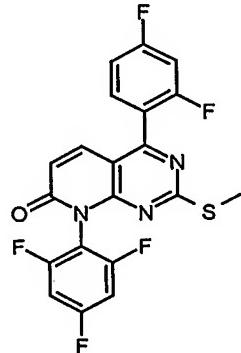
20

- b) 4-(2,4,6-trifluorophenylamino)-6-(2,4-di-fluorophenyl)-2-methylsulfanyl-pyrimidine-5-carbaldehyde



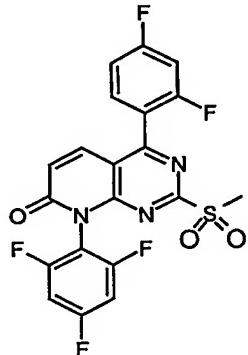
To the product of the preceding example of step (a) (1.3 g, 3.9 mmol) in dioxane (45 mL) and H₂O (15 mL) was added anhydrous K₂CO₃ (1.62 g, 11.7 mmol) followed by phenylboronic acid (0.93 g, 5.86 mmol, 1.5 eq). The reaction mixture was degassed
5 by bubbling a stream of Ar through the solution for 10 min and then tetrakis(triphenylphosphine)-palladium (225.6 mg, 0.195 mmol, 0.05 eq) was added. The reaction mixture was heated under reflux for 20 h, cooled 23°, the layers were separated. EtOAc (100 mL), followed by satd aq NaCl solution (100 mL), was added, the organic layer was separated and dried (MgSO₄), filtered and the yellow
10 solution was concentrated under reduced pressure. The residue was purified by flash chromatography (Hex/AcOEt = 95/5) to afford 1.1 g (67 % yield) of the title compound. LC MS (m/e) = 412.2 (MH⁺).

c) 8-(2,4,6-Trifluoro-phenyl)-4-(2,4-difluorophenyl)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one
15



To a solution of the product of the preceding example, step (b) (1.1 g, 2.67 mmol) in pyridine (10 mL) was added Ac₂O (10 mL) and the reaction mixture was heated under reflux for 64 hours, concentrated under reduced pressure and the residue was dissolved in EtOAc (200 mL), washed with 1 M aq Na₂CO₃, and H₂O and satd aq NaCl, dried (MgSO₄), filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography (Hex/AcOEt = 90/10) to afford pure 4,8-bis-(2-fluoro-phenyl)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one (0.93 g, 80 % yield). LC MS (m/e) = 436.2 (MH⁺).

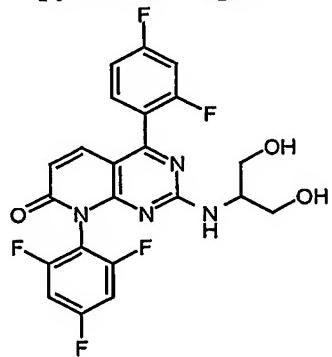
d) 8-(2,4,6-Trifluoro-phenyl)-4-(2,4-difluorophenyl)-2-methylsulfonyl-8H-pyrido[2,3-d]pyrimidin-7-one



5 The product of the preceding example, step (c) (0.93 g, 2.13 mmol) in CH₂Cl₂ (20 mL) was added 3-chloro-peroxybenzoic acid (0.96 g, 4.3 mmol, 77% purity) and the reaction mixture was stirred 1 h at 23°, 0.75 ml Me₂S was added to quench the reaction. Then 1 M aq Na₂CO₃ (20 mL) was added, the layers were separated, and the organic layer was washed with H₂O, dried (MgSO₄) and concentrated under reduced pressure. The yellow residue was purified by flash chromatography (Hex/AcOEt = 60/40) to afford 4,8-bis-(2-chloro-phenyl)-2-methanesulfonyl-8H-pyrido[2,3-d]pyrimidin-7-one (0.95 g, 95 % yield) to afford the title compound. LC MS (m/e) = 468.0 (MH⁺).

10

15 e) 8-(2,4,6-Trifluoro-phenyl)-4-(2,4-difluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one

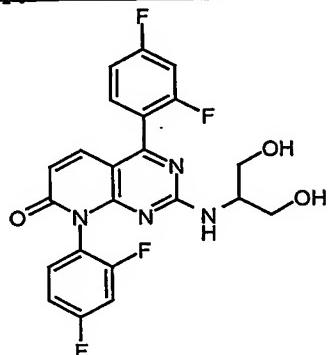


The product of the preceding example (0.95g, 2.03 mmol) in 1-methyl-2-pyrrolidinone (15 mL) was added serinol (0.925 g, 10.15 mmol) and the reaction 20 mixture was stirred at 23°. After 14 h, H₂O (60 mL) was added, followed EtOAc (60 mL). The layers were separated. The organic layer was washed with satd aq NaCl, dried (MgSO₄), filtered concentrated under reduced pressure. The yellow residue was then purified by flash chromatography to afford (0.93 g, 96 % yield) of title compound. LC MS (m/e) = 479 (MH⁺) 1.72 (Rt, min).

Example 2

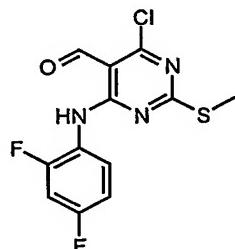
8-(2,4, difluorophenyl)-4-(2,4-di-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-

5 ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



a) 4-Chloro-6-(2,4-difluorophenylamino)-2-methylsulfanyl-pyrimidine-5-

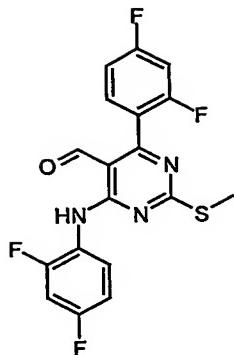
10 carbaldehyde



To a solution of 4,6-dichloro-2-methylsulfanyl-pyrimidine-5-carbaldehyde [Santilli, et al., *J. Heterocycl. Chem.* 1971, 8, 445-45] (4.0 g, 18.0 mmol) in CHCl₃ (50 mL) was added 2,4-di-fluoroaniline (2.02 mL, 19.8 mmol) followed by Et₃N (3.76 mL, 27 mmol,

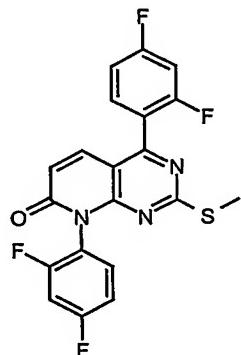
15 1.5 eq). The reaction mixture turned yellow and was heated to reflux for 6 h, H₂O (50 mL) was added and the layers were separated. The organic layer was evaporated to give 6.5 g (> 100 %) of the crude title compound which is pure enough to be used in the next step. LC MS (m/e) = 316 (MH⁺).

20 b) 4-(2,4-difluorophenylamino)-6-(2,4-di-fluorophenyl)-2-methylsulfanyl-pyrimidine-5-carbaldehyde



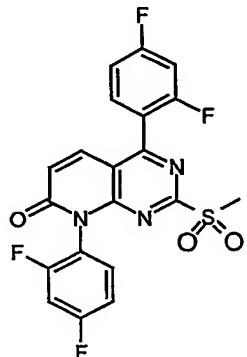
To the product of the preceding example, step (a) (5.67 g, 18 mmol) in dioxane (150 mL) and H₂O (50 mL) was added anhydrous K₂CO₃ (7.47 g, 54 mmol) followed by 2,4-difluorophenylboronic acid (3.41 g, 21.6 mmol, 1.2 eq). The reaction mixture
5 was degassed by bubbling a stream of Ar through the solution for 10 min. and then tetrakis(triphenylphosphine)-palladium (1.03 g, 0.90 mmol, 0.05 eq) was added. The reaction mixture was heated under reflux for 12 h, cooled to 23°, the solvents were removed, EtOAc (400 mL), followed by H₂O (200 mL), was added, the organic layer was separated. The organic phase was washed with satd aq NaCl, dried
10 (MgSO₄), filtered and the yellow solution was concentrated under reduced pressure to afford 7.75 g (>100 % crude yield) of the title compound which can be used directly in the next step. LC MS (m/e) = 394 (MH⁺).

c) 8-(2,4-difluorophenyl)-4-(2,4-difluorophenyl)-2-methylsulfanyl-8H-
15 pyrido[2,3-d]pyrimidin-7-one



To a solution of the product of the preceding example, step (b) (2.33 g, 5.9 mmol) in pyridine (15 mL) was added Ac₂O (15 mL) and the reaction mixture was heated under reflux for 48 h, concentrated under reduced pressure and the residue was dissolved in EtOAc (200 mL), washed with 1 M aq Na₂CO₃ twice, and H₂O and satd aq NaCl, dried (MgSO₄), filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography to afford the title compound
20 (1.2 g, 48 % yield for three steps). LC MS (m/e) = 418 (MH⁺).

- d) 8-(2,4-difluorophenyl)-4-(2,4-difluorophenyl)-2-methylsulfonyl-8H-pyrido[2,3-d]pyrimidin-7-one

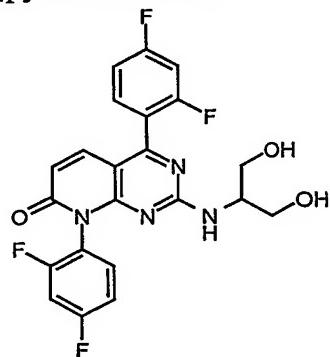


To the product of the preceding example, step (c) (2.0 g, 4.8 mmol) in CHCl₃ (60

5 mL) was added 3-chloro-peroxybenzoic acid (2.48 g, 14.3 mmol) and the reaction mixture was stirred 5 h at 23°, then 1 M aq Na₂CO₃ (100 mL) was added, the layers were separated, and the organic layer was washed with H₂O, dried (MgSO₄) and concentrated under reduced pressure to afford (2.02 g, 94 % yield) of the title compound. LC MS (m/e) = 450 (MH⁺).

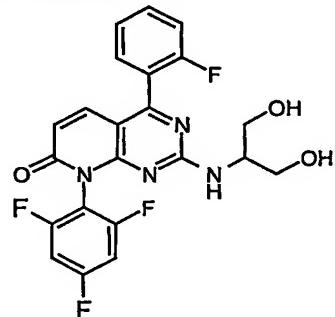
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- e) 8-(2,4-difluorophenyl)-4-(2,4-difluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one

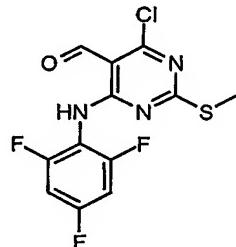


To the product of the preceding example, step (d) (1.90g, 4.3 mmol) in 1-methyl-2-

15 pyrrolidinone (60 mL) was added serinol (1.97g, 21.7 mmol) and the reaction mixture was heated to 50°. After 1 h, H₂O (100 mL) was added, followed by Et₂O (100 mL) and EtOAc (100 mL). The layers were separated. The organic layer was washed with satd aq NaCl, dried (MgSO₄), filtered concentrated under reduced pressure. The yellow residue was purified by Flash chromatography to afford (1.22 g, 62 % yield) of title compound. LC MS (m/e) = 461 (MH⁺) 1.62 (Rt, min).

Example 3**8-(2,4,6-Trifluorophenyl)-4-(2-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one**

- 5 a) 4-Chloro-6-(2,4,6-trifluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde



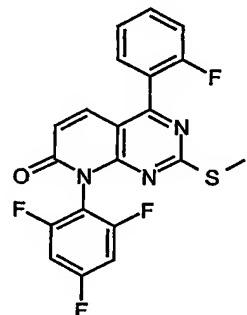
A solution of 4,6-dichloro-2-methylsulfanyl-pyrimidine-5-carbaldehyde [Santilli, et al., *J. Heterocycl. Chem.* **1971**, 8, 445-45] (1.0 g, 4.48 mmol) in CHCl₃ (20 mL) was added
10 2,4,6-trifluoroaniline (0.735 mg, 5 mmol) followed by Et₃N (0.94 mL, 6.72 mmol, 1.5 eq). The reaction mixture turned yellow and was heated to 50°C for 8 h, 1 M aq Na₂CO₃ (50 mL) was added and the layers were separated. The organic layer was washed with 50 mL satd aq NaCl solution, dried through anhydrous MgSO₄ and evaporated. The crude product was purified by flash chromatography to afford 1.3 g (87 %) of pure 4-chloro-6-
15 (2,4,6-trifluorophenylamino)-2-methylsulfanyl-pyrimidine-5-carbaldehyde. LC MS (m/e) = 334.0 (MH⁺).

- b) 4-(2,4,6-Trifluoro-phenylamino)-6-(2,4-di-fluorophenyl)-2-methylsulfanyl-pyrimidine-5-carbaldehyde



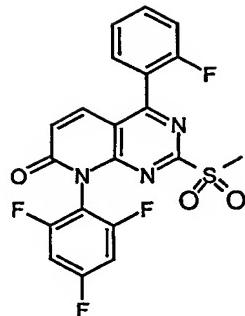
To the product of the preceding example, step (a) (0.89 g, 2.67 mmol) in dioxane (30 mL) and H₂O (10 mL) was added anhydrous K₂CO₃ (1.11 g, 8.03 mmol) followed by 2-fluorophenylboronic acid (0.56 g, 4.0 mmol, 1.5 eq). The reaction mixture was degassed by bubbling a stream of Ar through the solution for 10 min
 5 and then tetrakis(triphenylphosphine)-palladium (154 mg, 0.134 mmol, 0.05 eq) was added. The reaction mixture was heated under reflux for 20 h, cooled to 23°, the layers were separated, EtOAc (100 mL), followed by satd aq NaCl solution (100 mL), was added, the organic layer was separated and dried (MgSO₄), filtered and the yellow solution was concentrated under reduced pressure. The residue was purified
 10 by flash chromatography to afford 530 mg (50 % yield) of the title compound. LC MS (m/e) = 394.0 (MH+).

c) 8-(2,4,6-Trifluoro-phenyl)-4-(2-fluorophenyl)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one



15 To a solution of the product of the preceding example, step (b) (530 mg, 1.35 mmol) in pyridine (6 mL) was added Ac₂O (6 mL) and the reaction mixture was heated under reflux for 64 h, concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL), washed with 1 M aq Na₂CO₃, and H₂O and satd aq
 20 NaCl, dried (MgSO₄), filtered and concentrated under reduced pressure. The yellow residue was purified by flash chromatography to afford pure 4,8-bis-(2-fluoro-phenyl)-2-methylsulfanyl-8H-pyrido[2,3-d]pyrimidin-7-one (520 mg, 92 % yield). LC MS (m/e) = 418.2 (MH+).

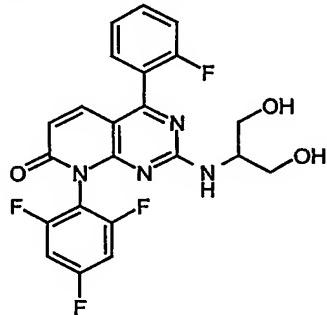
25 d) 8-(2,4,6-Trifluoro-phenyl)-4-(2-fluorophenyl)-2-methylsulfonyl-8H-pyrido[2,3-d]pyrimidin-7-one



The product of the preceding example, step (c) (520 mg, 1.24 mmol) in CH_2Cl_2 (15 mL) was added 3-chloro-peroxybenzoic acid (555 mg, 2.48 mmol, 77% purity) and the reaction mixture was stirred 1 h at 23° , 0.5 mL Me_2S was added to quench the reaction. Then 1 M aq Na_2CO_3 (50 mL) was added, the layers were separated, and the organic layer was washed with H_2O , dried (MgSO_4) and concentrated under reduced pressure to afford 4,8-bis-(2-chloro-phenyl)-2-methanesulfonyl-8H-pyrido[2,3-d]pyrimidin-7-one (470 mg, 84 % yield) to afford the title compound. LC MS (m/e) = 449.8 (MH^+).

10

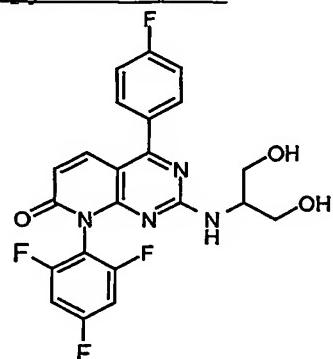
- e) 8-(2,4,6-trifluorophenyl)-4-(2-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



The product of the preceding example, step (d) (135 mg, 0.3 mmol) in 1-methyl-2-pyrrolidinone (2 mL) was added serinol (137 mg, 1.5 mmol) and the reaction mixture was stirred at 23° . After 14 h, H_2O (30 mL) was added, followed by EtOAc (30 mL). The layers were separated. The organic layer was washed with satd aq NaCl , dried (MgSO_4), filtered concentrated under reduced pressure. The yellow residue was then purified by Flash chromatography to afford (120 mg, 87 % yield) of title compound. LC MS (m/e) = 461 (MH^+) 1.65 (Rt, min).

Example 4

8-(2,4,6-Trifluorophenyl)-4-(4-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



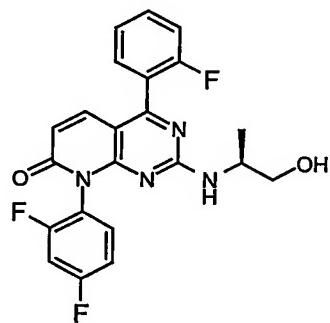
5

This compound was prepared using the methods of example 1 herein, and substituting 4-fluorophenyl boronic acid in the procedure of example 1(b) to afford the title compound. LC-MS: 461 (MH+, m/z), 1.69 (Rt, min).

10

Example 5

8-(2,4-difluorophenyl)-4-(2-fluorophenyl)-2-((S)-2-hydroxy-1-methylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



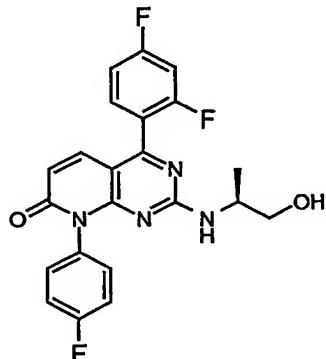
15

This compound was prepared using the methods of example 2, and substituting 2-fluorophenyl boronic acid in the procedure of example 2(b) and (S)-(+)-2-amino-1-propanol in the procedure of example 2(e) to afford the title compound. LC-MS: 427 (MH+, m/z), 1.82 (Rt, min).

20

Example 6

8-(4-fluorophenyl)-4-(2,4-difluorophenyl)-2-((S)-2-hydroxy-1-methylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



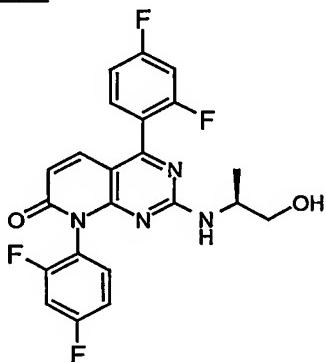
5

This compound was prepared using the methods of example 2, and substituting 4-fluoroaniline in the procedure of example 2(a) and (S)-(+)-2-amino-1-propanol in the procedure of example 2(e) to afford the title compound. LC-MS: 427 (MH+, m/z), 1.87 (Rt, min).

10

Example 7

8-(2,4-difluorophenyl)-4-(2,4-difluorophenyl)-2-((S)-2-hydroxy-1-methylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



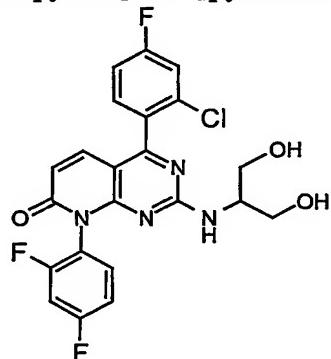
15

This compound was prepared using the methods of example 2, and substituting 2,4-difluoroaniline in the procedure of example 2(a), and substituting 2,4-difluorophenyl boronic acid in the procedure of example 2(b), and (S)-(+)-2-amino-1-propanol in the procedure of example 2(e) to afford the title compound.

20 LC-MS: 445 (MH+, m/z), 1.95 (Rt, min).

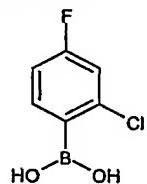
Example 8

8-(2,4-Difluorophenyl)-4-(2-chloro-4-fluorophenyl)-2-(2-hydroxy-1-hydroxymethylethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



5

a) 2-chloro-4-fluorophenylboronic acid

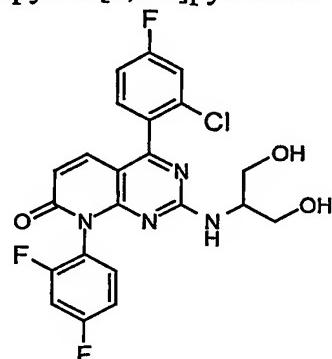


To 1.05 g Mg in THF (20 mL) was added catalytic I₂ and then a 1 mL portion of the 2-Chloro-4-fluoro-iodobenzene (10g total, 39 mmol) was added. The mixture was

10 stirred, slowly warmed, and an exothermic reaction began. The remainder of the 2-Chloro-4-fluoro-iodobenzene was added at a rate to maintain the exotherm and then the reaction was heated to THF reflux for 2.5 h, then cooled to 23°. The resulting mixture was added with rapid stirring to a -70° solution of trimethyl borate (4.53 mL, 40 mmol) in THF (40 mL). The reaction was stirred at < 0° for 20 min and at 23° for 16 h, then 15 poured into 2N HCl (150 mL), stirred 2 h, and the THF was removed under vacuum. The residual aqueous solution was extracted with Et₂O (3 X 200 mL) and the combined extracts were dried (Na₂SO₄) and concentrated to afford a red solid. Trituration with hexane and drying afforded the title compound as a tan solid. (1.89 g, 28%). . ¹H-NMR CD₃OD δ 7.09 (m, 1H), 7.21 (m, 1H), 7.38 (m, 1H).

20

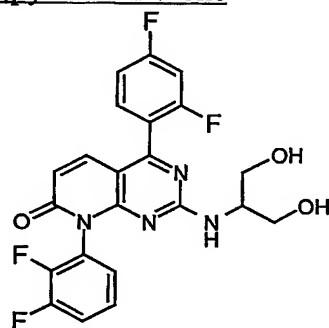
b) 8-(2,4-difluorophenyl)-4-(2-chloro-4-fluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



- 5 The title compound was prepared using the methods of example 2 and substituting the product of the preceding example in the procedure of example 2(b), to afford the title compound. LC-MS: 477 (MH⁺, m/z), 1.75 (Rt, min).

Example 9

- 10 8-(2,3-difluorophenyl)-4-(2,4-difluorophenyl)-2-(2-hydroxy-1-hydroxymethyl-ethylamino)-8H-pyrido[2,3-d]pyrimidin-7-one



- This compound was prepared using the methods of example 2 and substituting 2,3-difluoroaniline in the procedure of example 2(a), to afford the title compound. LC-MS: 461 (MH⁺, m/z), 1.67 (Rt, min).

20 All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding

description, utilize the present invention to its fullest extent. Therefore, the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.